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Zh. V. Vasilieva, E. Yu. Moshnyatckaya

Extraction of bioflocculants from activated sludge and their application to wastewater treatment

Extracellular polymeric substances (EPS) – biopolymers produced by the microorganisms – are effective flocculants of wastewater pollution and lack the shortcomings of traditional coagulants and flocculants, which can pose direct threat to health and human life, as well as to the sustainable existence of aquatic and terrestrial ecosystems. EPS do not form secondary contamination of their degradation intermediates, are biodegradable and eco-friendly. Industrial production of bacterial EPS is associated with high cost of growing specific microbial biomass and the functioning of technologies for the synthesis of microbial products. At the same time, there is an underused resource of excess activated sludge, which can be used as cheap substrate for producing bioflocculants and a possible measure to reduce costs. The conducted researches have shown the prospects of extracting EPS from excess activated sludge for their subsequent use as wastewater treatment bioflocculants. EPS extraction has been conducted using three methods: combination of centrifugation processes, extraction using the aqueous solution of disodium ethylenediaminetetraacetic acid, and precipitation with isopropyl alcohol (the EDTA method); combination of centrifugation, extraction with $(NH_2)_2CO$, precipitation and ethanol reprecipitation (the $(NH_2)_2CO$ method); combination of activated sludge ultrasonic treatment, centrifugation, extraction with glacial acetic acid, and precipitation with acetone (the CH_3COOH method). The research has shown that the extraction method affects not only the efficiency of EPS extraction, but also the possibility of EPS application for the purification of certain types of sewage. The $(NH_2)_2CO$ method has shown the best extraction efficiency, but at the same time EPSs produced have not be able to perform fish processing wastewater treatment. The EDTA and CH_3COOH methods are more preferable for producing efficient bioflocculants for fish processing wastewater treatment. The use of EPS obtained by the EDTA method has resulted in the significant reduction of total suspended solids and optical density; EPS produced by the CH_3COOH method have reduced the content of dissolved solids and the optical density. According to the conducted research EPS produced from surplus sludge with the EDTA and CH_3COOH methods have good flocculation and are harmless for humans and the environment, and thus may be a potential substitute for traditional synthetic flocculants and could be used in the treatment of fish processing plants wastewaters.

Key words: extracellular polymeric substances (EPS), bioflocculants, activated sludge, wastewater treatment.

Introduction

Coagulation and flocculation have received a wide spread in the field of industrial wastewater treatment, this is due to their following advantages: the possibility of treating wastewater to the required values from virtually all types of contaminants that differ in both chemical and phase-dispersed composition; technological flexibility of cleaning systems when changing the parameters of incoming water; rational size of treatment facilities, no need for large production areas, etc. However, the experience of using traditional synthetic flocculants and coagulants in wastewater treatment has revealed a number of their significant shortcomings: the formation of neurotoxic and carcinogenic acrylamide monomers of flocculants as well as high residual content of heavy metal ions (Al^{3+}, Fe^{3+}) of coagulants in the water. The links have been revealed between the existence of Al in the drinking water and human neurological disorders such as dialysis encephalopathy [1]. Along with this, the evidence of links in case of Alzheimer's disease caused by aluminum salts have been determined [2]. The arguments associated with the potential danger of effects of acrylamide monomers on the environment and human health have been summarized [3; 4]. These shortcomings do not limit or exclude the use of synthetic flocculants and coagulants, which can pose a direct threat to the health and human life, and a threat to the sustainable existence of aquatic and terrestrial ecosystems.

On the other hand, other agents of wastewater treatment have become known in recent years – extracellular polymeric substances (EPS). Extracellular polymeric substances, or as it is called, microbial bioflocculants, do not form a secondary contamination of their degradation intermediates; they are biodegradable and eco-friendly [5–8]. EPS are functionally flocculating agents that promote agglomeration of dispersion and colloidal particles of wastewater into well precipitating complexes; therefore they are capable to achieve a significant reduction of pollutants concentrations contained in the wastewater [9; 10].

The production of microbial EPS in Russia does not meet the requirements of the increasing demand of various industries, medicine and agriculture in these biopolymers [11]. Meanwhile, industrial production of bacterial EPS is associated with the high cost of growing specific microbial biomass, the functioning of technologies for the synthesis of microbial products.

At the same time, there is an obvious resource for obtaining significant amounts of microorganism biomass and products of their synthesis. Biological treatment facilities daily produce a significant amount of excess activated sludge, which is a consortium of microorganisms of various systematic groups of micromycetes, yeasts, and bacteria, which in their turn are natural producers of exobiopolymers. At present, this resource is a large-scale waste and does not find suitable practical application. Thus, activated sludge can be used as a cheap substrate for the production of bioflocculants as a possible measure to reduce costs.

In the scope of this work studies have been conducted that have shown the prospect of extracting EPS from excess activated sludge for their subsequent use as bioflocculants of wastewater treatment. In addition, until now there has been no report about treating fish processing wastewater using bioflocculants. Accordingly, EPS extraction from excess activated sludge and investigating the effects of bioflocculant extraction methods on the efficiency of fish processing wastewater treatment have been the aims of this study.

Materials and methods

Samples of excess activated sludge were collected from the wastewater treatment system at the JSC "Murmansk Bakery Complex" in the city of Murmansk, the Murmansk region, Russia. Activated sludge was collected from the aeration tank. The collected samples were transported to the laboratory within 2 hours after sampling at the temperature of 4 °C. Determination of hydro-chemical parameters of activated sludge was carried out in accordance with the methodics[1]. The basic properties of the sludge water are listed in Table 1.

Table 1. Characteristics of activated sludge
Таблица 1. Показатели активного ила

	Suspended Solids, g/l	Sludge Volume Index	Settled Sludge Volume, ml/l	pH	Temperature, °C
Activated sludge samples	2.3	65	150	7.1	8.5

Extraction of EPS has been conducted using three different methods:

– the EDTA method – a combination of centrifugation processes, extraction using aqueous solution of disodium ethylenediaminetetraacetic acid (EDTA), and precipitation of isopropyl alcohol [12];

– the $(NH_2)_2CO$ method – a combination of centrifugation, extraction with $(NH_2)_2CO$, precipitation and ethanol reprecipitation [13];

– the CH_3COOH method – a combination of ultrasonic cavitation treatment of activated sludge, centrifugation, extraction with glacial acetic acid, and precipitation with acetone [14].

The composition of the swollen biopolymers has been studied with the method described in [15], using the microscope LOMO Mikmed-2 in the visible luminescence light, when illuminated from above with light exciting the luminescence. Pre-coloring of EPS preparations has been carried out with fluorochrome acridine orange [16].

The study on flocculation properties of microbial EPS preparations has been carried out within the process of wastewater chemical treatment, which includes dosing, mixing of extracted EPS with wastewater, flocculation, and deposition. Representative samples for laboratory study have been collected from the seafood-processing plant "Port Vladimir". Efficiency of wastewater treatment has been evaluated in ratio of total suspended solids, dissolved solids, and optical density in raw untreated and treated wastewater in accordance with standard methods[2]. The flocculating effect has been measured at 540 nm by determining the optical density of the treated water after flocculation for 100 minutes (n) with respect to the optical density of the raw untreated wastewater (n_0) according to the following equation:

$$\textit{Flocculating effect}\ (\%) = \frac{n_0 - n}{n_0}.$$

Results and discussion

Extraction efficiency is an important technological parameter, which makes it possible to determine the feasibility of selecting and implementing the most effective technology for extracting EPS in the future. The amount of EPS obtained in this study is shown in Table 2. The maximum extraction efficiency is shown by the

[1] A set of methods for hydrochemical control of activated sludge: determination of the mass concentration of activated sludge, sludge index, ash content of raw sludge, activated sludge, transparency of nadil water: federal register (FR): FR 1.31.2008.04397, FR 1.31.2008.04398, FR 1.31.2008.04399, 1.31.2008.04400. M. : AKVAROS, 2008. 37 p. (in Russian)

[2] Quantitative chemical analysis of waters. The method 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. M., 1997. 7 p. (in Russian) ; Quantitative chemical analysis of waters. The method for performing measurements of the mass concentration of dry residue in samples of natural and treated wastewater by the gravimetric method : HDPE F 14.1:2.114-97. M., 1997. 6 p. (in Russian)

$(NH_2)_2CO$ method, followed by the EDTA method, the lowest efficiency is shown by the extraction method CH_3COOH.

Table 2. EPS extraction efficiency (n = 10)
Таблица 2. Эффективность экстракции ВПВ (n = 10)

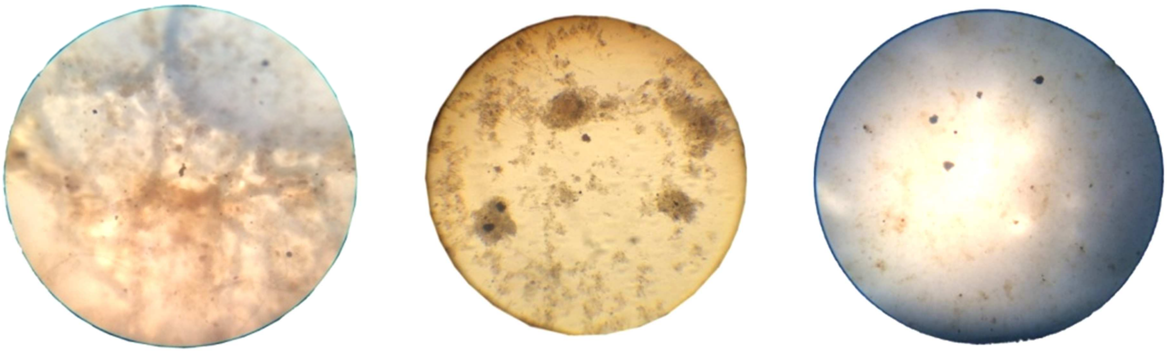
Property	The EDTA method	The $(NH_2)_2CO$ method	The CH_3COOH method
Extraction efficiency (converted by the suspended solids concentration of activated sludge), %	13.08 ± 3.57	15.2 ± 4.37	5.22 ± 2.79

The resulting dry preparations of EPS are powder which varies from light gray or brown to dark green color depending on the technology. Fig. 1 shows EPS samples obtained via the three approved technologies.

a *b* *c*

Fig. 1. Samples of EPS (dry) obtained via three technologies: *a* – the EDTA method; *b* – the $(NH_2)_2CO$ method; *c* – the CH_3COOH method
Рис. 1. Образцы ВПВ (сухие), полученные по технологиям: *a* – метод EDTA; *b* – метод $(NH_2)_2CO$; *c* – метод CH_3COOH

The study of the swollen biopolymers composition is shown in Fig. 2. The bioflocculant obtained with the EDTA method has a mucous, stretch-like structure resembling another natural bioflocculant – gelatin; the structure of the biopolymer obtained with the $(NH_2)_2CO$ method has the form of cotton particles. EPS received via the CH_3COOH method have a granular structure.

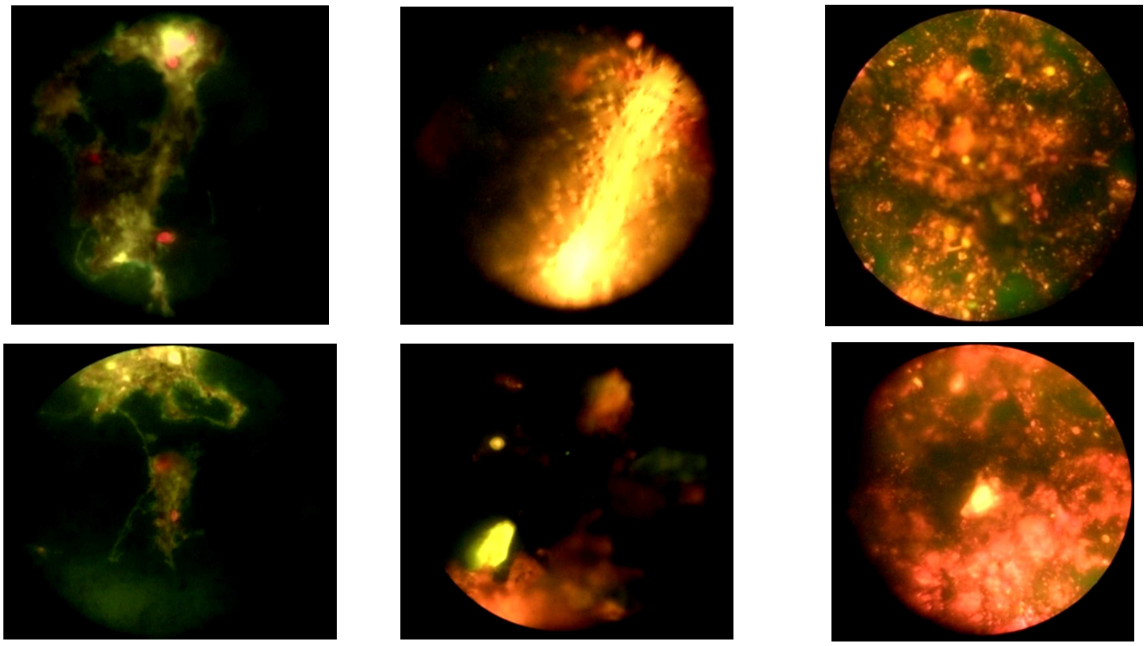


a *b* *c*

Fig. 2. Samples of EPS (swollen) obtained due to the three approved technologies: *a* – the EDTA method; *b* – the $(NH_2)_2CO$ method; *c* – the CH_3COOH method
Рис. 2. Образцы ВПВ (набухшие), полученные по технологиям: *a* – метод EDTA; *b* – метод $(NH_2)_2CO$; *c* – метод CH_3COOH

The resulting bioflocculants are pre-colored with fluorochrome acridine orange under illumination with ultraviolet rays through the green filter, and have shown the red and green fluorescence (Fig. 3). This suggests that EPS contain mainly polysaccharides (red luminescence) and protein structures (green luminescence) of different functional groups in their composition. In this case, the samples obtained with the EDTA method have contained mainly proteins with small impregnations of polysaccharides, and in contrast, the samples obtained with the CH_3COOH method have contained higher quantities of polysaccharide.

Evaluation of EPS flocculating properties has shown the ambiguity of the results of wastewater treatment efficiency (Table 3). Results of the study have indicated that only the bioflocculants obtained due to the EDTA and CH_3COOH methods purified the wastewater effectively.



a *b* *c*

Fig. 3. Fluorescent staining images of EPS: *a* – the EDTA method; *b* – the $(NH_2)_2CO$ method; *c* – the CH_3COOH method
Рис. 3. Флуоресцентные изображения структуры ВПВ: *a* – метод EDTA; *b* – метод $(NH_2)_2CO$; *c* – метод CH_3COOH

It should be noted that in the classical case, the reagent treatment of wastewater is mainly aimed at reducing the values of suspended matter and optical density (or turbidity). Reduction of these criteria certainly leads to a decrease in the associated indicators of the content of phosphorus, nitrogen, BOD, COD and other components. For example, wastewater treatment with bioflocculants obtained using the EDTA method is a classic case of reagent treatment with the purification efficiency on the optical density of 27.97 ± 6.1 %, and on the indicator on total suspended solids of 25.78 ± 3.45 %.

Bioflocculants obtained with the CH_3COOH method had the best efficiency of wastewater treatment with respect to the optical density indices – 42.43 ± 6.25 %. It is interesting to note, that in all series of experiments, this type of bioflocculants significantly has reduced the amount of dissolved solids in the wastewater, which is untypical for classical reagents. Thus, the decrease in the content of dissolved solids is 23.35 ± 10.84 %. The result of the purification effectiveness of total suspended solids – 0.1 % – indicates that this type of bioflocculant is poorly "working" with respect to this indicator, which is probably due to the following reasons: either the bioflocculant dose is too small to reduce this indicator, or it is not suitable for this type of wastewater in relation to this indicator. The bioflocculant obtained with the EDTA method purifies wastewater most effectively from total suspended solids. Bioflocculant obtained via the $(NH_2)_2CO$ method, on the contrary, introduces a significant amount of suspended matter to the wastewater.

The use of EPS obtained with the technological method $(NH_2)_2CO$ is recognized by the authors as inexpedient for the purification of wastewater from fish processing plants, since all the investigated purification indexes have been unsatisfactory. However, it does not mean that this bioflocculant will not work with other kinds of wastewater, as it happens with most industrial flocculants (and some coagulants) that can "work" only for specific types of wastewater.

Table 3. Efficiency of wastewater treatment by EPS
Таблица 3. Эффективность очистки сточных вод с помощью биофлокулянтов

Constituent	Treatment efficiency, %		
	The EDTA method	The $(NH_2)_2CO$ method	The CH_3COOH method
Total suspended solids	25.78 ± 3.45	0.3 ± 0.24	0.1 ± 0.0
Optical density	27.97 ± 6.1	–0.3 ± 5.19	42.43 ± 6.25
Dissolved solids	–3.6 ± 2.7	–4.8 ± 5.6	23.35 ± 10.84

The next stage of work has been determined by the need to establish the feasibility of increasing bioflocculants dose to improve the degree of wastewater treatment. It has been found out that in case of increasing the bioflocculant dose from 1 g/l to 10 g/l, efficiency of wastewater treatment is significantly increased as well (Table 4). Thus, the removal efficiencies of weighted substances has increased from 25.78 ± 3.45 to 74.21 ± 0.0 %, the removal efficiencies of turbidity – from 27.97 ± 6.1 to 69.46 ± 0.0 %.

Table 4. Improving the degree of wastewater treatment with increased bioflocculant dose (the EDTA method)
Таблица 4. Улучшение степени очистки сточных вод с увеличением дозы биофлокулянта, полученного методом EDTA

Constituent	Untreated wastewater	Treated wastewater by EPS			
		1 g/l		10 g/l	
		Concentration, mg/l	Treatment efficiency, %	Concentration, mg/l	Treatment efficiency, %
Total suspended solids, mg/l	620 ± 22.95	465 ± 30.89	25.78 ± 3.45	159.8 ± 27.66	74.21 ± 6.8
Optical density, l/cm	0.63 ± 0.34	0.46 ± 0.99	27.97 ± 6.1	0.19 ± 0.38	69.46 ± 2.0

Fig. 4 shows the dependencies of the wastewater optical density being purified and the biofloculating effect as a function of operating time. An analysis of the presented dependencies shows that a significant decrease of the optical density index occurs almost immediately, in the first 10–30 minutes from the bioflocculation starting point, but the optimal settling time is the time interval of about 100 minutes.

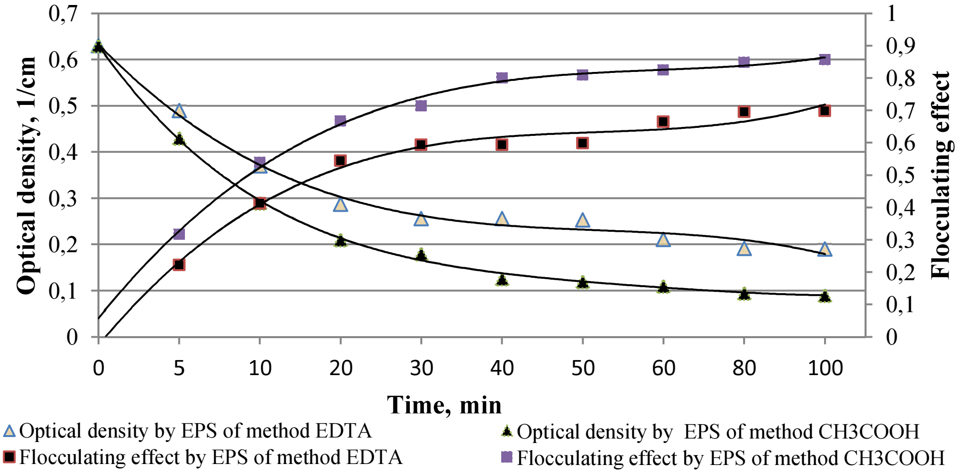


Fig. 4. Optical density and flocculating effect as a function of operating time
Рис. 4. Оптическая плотность и флокуляционный эффект в зависимости от времени

Conclusions

The extraction method affects not only the efficiency of EPS extraction, but also the possibility of EPS application for the purification of certain types of sewage. The $(NH_2)_2CO$ method has the highest extraction efficiency (15.2 ± 4.37 %), but produces EPS not capable of performing fish processing wastewater treatment. The EDTA and CH_3COOH methods are preferable for the production of effective wastewater bioflocculants for fish processing plants, although their extraction efficiency is slightly lower – 13.08 ± 3.57 and 5.22 ± 2.79 % respectively. The use of EPS obtained by the EDTA method leads to the significant reduction of total suspended solids and optical density; EPS got with the CH_3COOH method reduce the content of dissolved solids and optical density. Probably, it is promising to create a complex of bioflocculants that has the capabilities of EPS of the EDTA and CH_3COOH methods to increase the efficiency of fish processing wastewater treatment in relation to all the indicators studied.

Indicators of efficiency of wastewater purification have increased with increasing the bioflocculant dose. Thus, with increase in the dose of bioflocculant obtained due to the EDTA method from 1 g/l to 10 g/l, the removal of suspended matter has increased from 25.78 ± 3.45 to 74.21 ± 6.8 %, and the flocculation effect – from 27.97 ± 6.1 to 69.46 ± 2.0 %.

According to the research conducted, the EPS obtained from active sludge with the EDTA and CH_3COOH methods have good flocculation and are harmless for humans and the environment, and thus may be a potential substitute for traditional synthetic flocculants in the wastewater treatment of fish processing plants.

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Information about authors

Vasilieva Zh. V. – 13, Sportivnaya Str., Murmansk, Russia, 183010; Murmansk State Technical University, Cand. of Tech. Sci., Associate Professor; e-mail: kuchugura@mail.ru

Васильева Жанна Вячеславовна – ул. Спортивная, 13, г. Мурманск, Россия, 183010; Мурманский государственный технический университет, канд. техн. наук, доцент; e-mail: kuchugura@mail.ru

Moshnyatckaya E. Yu. – 13, Sportivnaya Str., Murmansk, Russia, 183010; Murmansk State Technical University, Undergraduate Student; e-mail: zhenyasha-1@mail.ru

Мошняцкая Евгения Юрьевна – ул. Спортивная, 13, г. Мурманск, Россия, 183010; Мурманский государственный технический университет, магистрант; e-mail: zhenyasha-1@mail.ru

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Ж. В. Васильева, Е. Ю. Мошняцкая

Получение биофлокулянтов из биомассы активного ила и использование их для очистки сточных вод

Внеклеточные полимерные вещества (ВПВ) – биополимеры, продуцируемые микроорганизмами – являются эффективными биофлокулянтами загрязнений сточных вод и лишены недостатков, присущих традиционным коагулянтам и флокулянтам, которые могут представлять как прямую угрозу для здоровья и жизни человека, так и угрозу устойчивому существованию водных и наземных экосистем. ВПВ не образуют вторичного загрязнения промежуточными продуктами гидролиза, являются биодеградируемыми и экологически чистыми. Промышленное производство ВПВ связано с высокой стоимостью выращивания специфической микробной биомассы, функционированием технологий синтеза микробных продуктов. В то же время существует такой недоиспользуемый ресурс, как избыточный активный ил, который можно применять в качестве дешевого субстрата для производства биофлокулянтов. В рамках работы были проведены исследования, которые показали перспективность экстракции ВПВ из активного ила для последующего использования их в качестве биофлокулянтов очистки сточных вод. Экстракция ВПВ проводилась с использованием трех методов: комбинации процессов центрифугирования, экстракции с помощью водного раствора динатрийэтилендиаминтетрауксусной кислоты, осаждения ВПВ изопропиловым спиртом (метод EDTA); комбинации центрифугирования, экстракции мочевиной, осаждения и переосаждения ВПВ этанолом (метод $(NH_2)_2CO$); комбинации процессов ультразвуковой обработки активного ила, центрифугирования, экстракции ледяной уксусной кислотой, осаждения ВПВ ацетоном (метод CH_3COOH). Исследования показали, что метод экстракции влияет не только на эффективность извлечения ВПВ, но и на возможность применения полученных ВПВ для очистки определенных типов сточных вод. Метод $(NH_2)_2CO$ показал наилучшую эффективность экстракции, но при этом продуцировались ВПВ, не способные выполнять обработку сточных вод рыбоперерабатывающих производств. Способы EDTA и CH_3COOH более предпочтительны для получения эффективных биофлокулянтов сточных вод рыбоперерабатывающих предприятий. Использование ВПВ, полученных методом EDTA, приводило к значительному уменьшению содержания взвешенных веществ и оптической плотности; применение ВПВ, полученных методом CH_3COOH, снижало содержание растворенных веществ и оптическую плотность. Согласно проведенному исследованию ВПВ, полученные из активного ила методами EDTA и CH_3COOH, обладают хорошими флокуляционными свойствами, безвредностью для человека и окружающей среды, потенциально могут служить заменителями традиционных синтетических флокулянтов и использоваться при обработке сточных вод.

Ключевые слова: внеклеточные полимерные вещества (ВПВ), биофлокулянты, активный ил, сточные воды.