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Research Article

Study of the polysaccharide production by the microalgae C-1509 *Nannochloris* sp. *Naumann*

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ABSTRACT

Biologically active compounds, including polysaccharides isolated from microalgae, have various properties. Although *Nannochloropsis* spp. have the potential to produce secondary metabolites important for biotechnology, only a small part of the research on these microalgae has focused on their ability to produce polysaccharide fractions. This study aims to evaluate the physicochemical growth factors of *Nannochloropsis* spp. microalgae, which ensure the maximum accumulation of polysaccharides, as well as to optimize the parameters of polysaccharide extraction. The optimal nutrient medium composition was selected to maximize biomass and polysaccharide accumulation. The significance of selecting the extraction module and extraction temperature regime, as well as the cultivation conditions (temperature and active acidity value) is emphasized. Important chemical components of polysaccharides responsible for their biological activity were identified

Abbreviations

EPS	exopolysaccharides;
FTIR	Fourier transform infrared spectrometry;
GlcA	Glucuronic Acid;
Xyl	xylene;
Gal	gallic acid;
Fuc	fucoidan;
IPPAS IPP RAS	Institute of Plant Physiology of the Russian Academy of Sciences;
KFK	photoelectric concentration photometer;
PS	polysaccharides;
DNA	Deoxyribonucleic acid;
ATP	adenosine triphosphate;
GC	gas chromatography.

1. Introduction

Nannochloropsis spp. microalgae are members of the phylum *Heterokontophyta*, the class *Eustigmatophyceae*, and the family *Eustigmataceae* [1]. These microalgae are unable to synthesize chlorophyll *B* and C; they can only accumulate chlorophyll A. It is this property that distinguishes these microalgae from related species [2]. *Nannochloropsis* spp. are important microalgae for biotechnology and medicine because they can synthesize pigments (astaxanthin, zeaxanthin, and canthaxanthin, beta-carotene) [2], phytosterols (beta-sitosterol, stigmasterol), fatty acids, flavonoids, alkaloids etc. [3]. Previous reports have shown that phenolic and carathinoid extracts isolated from *Nannochloropsis* spp. species can inhibit pathogenic fungi of the genus *Fusarium* that cause animal and plant diseases [4]. The addition of *Nannochromiss* spp. to animal feed has been discussed in the scientific literature as a potential source of antioxidant compounds [5].

The *Nannochloropsis spp*. microalgae contain carotenoids, pigments,

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chlorophyll A, polysaccharides, essential amino acids, polyphenols, and minerals [6]. *Nannochloropsis* spp. accumulates a polysaccharide, β-d-glucoside, which has a branched structure due to the presence of 1, 3-β-glycosidic linkage and 1,6- β-glycosidic linkage. Exopolysaccharides (EPSs) are carbon and energy stores for cells that are often released by microalgae under stress conditions and are also produced through complex physiological processes. Stressful conditions include a lack of nitrogen and phosphorus in the nutrient medium. In laboratory conditions, EPSs are produced by low-temperature precipitation. Alcohol is used as a precipitant [7]. The efficiency of the precipitation method also depends on the type of alcohol used. Various polysaccharides can be isolated, each with its own set of technological properties such as heat resistance, stability, moisture retention, and water solubility. These properties of EPSs allow microalgae exopolysaccharides to be used in the medical, pharmaceutical, food, and cosmetic industries as hydrocolloids and stabilizers [8,9]. A previously described experiment demonstrated that bound polysaccharides can be isolated using a physical method, ultrasound [9]. Gas chromatography (GC), thermogravimetric analysis, and infrared spectrometry (FTIR) have been used to characterize microalgae exopolysaccharides (EPSs) [10–13].

Under unfavorable conditions (high salt concentrations in wastewater, CO_2 concentrations ranging from 5 % to 15 %, pH up to 4.0, temperature up to 40 °C), the microalgae *Nannochloropsis* spp. can accumulate biomass and grow rapidly while synthesizing a significant amount of lipids [14–18].

However, only a few studies are currently being conducted on the biotechnological production of polysaccharides from these microalgae [19–21]. Microalgae polysaccharides are biodegradable, biocompatible, and safe substances [22,23]. The anti-inflammatory, antioxidant, antimicrobial, and antitumor properties of microalgae are especially interesting [24]. The polysaccharides of *Nannochloropsis oculata* are rich in anionic sulfated heteroramnans, which contain monomers such as xyla, Rha, GlcA, Gal, and Fuc. It was also demonstrated that polysaccharides are represented by 1–3β,1–4β-glucans and 1–3α-,1–4α-mannans. These fractions have immunostimulatory properties [25]. Polysaccharides from *N. oculata* have been shown to have antimicrobial activity and antioxidant activity and three species of the *Candida genus*, anticancer activity, and anticholinesterase activity [26].

It is possible to increase the intensity of polysaccharide synthesis by microalgae by creating stress conditions and limiting nutrients [27]. Not all varieties of microalgae have a naturally high concentration of polysaccharides, necessitating the selection of these physicochemical factors. However, it is possible to adapt the metabolism of microalgae to produce more polysaccharides by changing the technological aspects of cultivation, such as changing temperature and light conditions, limiting the composition of macro- and microelements (primarily nitrogen), and salt stress. Thus, optimizing microalgae cultivation technological conditions leads to increase in the rate of cell growth, biomass production, and polysaccharide yield [28]. In accordance with this, the optimal medium parameters (temperature, active acidity, extraction time) and extraction of polysaccharides from *Nannochloropsis* spp. cells were selected in the study, the effect of salt deficiency in the nutrient medium on the intensity of biomass and polysaccharide accumulation was also evaluated.

2. Materials and methods

2.1. Microalgae cultivation

From the Collection of microalgae of the K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences *Nannochloris* sp. *Naumann* C-1509 strain was purchased. The process of cultivation and biomass production was performed in conical flasks (250 mL) at a temperature (15–30 °C) [29] under constant illumination with white light at an intensity of 80 mmol/m^2/*sec*. As a light source, 110 mm wide and 500 mm long LED lamps from Tetropnic LED ProLine 580 (Tetra, Melle, Germany) were used. The duration of cultivation was 7 days. The microalgae were cultivated on a liquid Zarrouk nutrient medium (Table 1).

The increase in microalgae biomass and cell concentration was evaluated every 3 days using a spectrophotometric method (at a wavelength of 750 nm) using a KFK-3KM spectrophotometer (Promyshlennye ekologichesiyel laboratorii, St. Petersburg, Russia) [30].

2.2. Alcohol precipitation of exopolysaccharides

Butanol was used as the water insoluble alcohol in the two-step extraction of exopolysaccharides. Medium samples with microbial cells were centrifuged (3900×*g*, 20 min) on a 1701 Hettich ROTINA 380 centrifuge (DV Ekspert, Moscow, Russia). The collected supernatant was filtered through a paper filter (Mirab, Moscow, Russia) with a pore size of 3 mm. Ethanol, butanol, or isopropanol were added to the filtrate in different ratios (1:1, 1:2, 1:3), and extraction was performed for 12 h at different temperatures (from −30 °C to +30 °C). After precipitation, the solution was centrifuged, and the supernatant was removed. Using an Iney-6 lyophilizer (IBF RAS, Pushchino, Russia), the precipitate was dried (temperature −20 °C, pressure 0.350 mbar, time 12 h). The amount of polysaccharide obtained after drying was determined gravimetrically. The mass of isolated polysaccharide was calculated using the formula

$$m = m_{\mathrm{OD480}}/m_{d.w.}, \quad (1)$$

where m_{OD480} – mass of isolated polysaccharides at an optical density of 480 nm,

$m_{d.w.}$ – polysaccharide mass per dry biomass of microalgae.

2.3. Ultrasonic extraction of endopolysaccharides

Cell wall destruction was performed using ultrasound. The ultrasound treatment parameters (power 20, 40, 60 W, time 1.0, 3.0, 4.0, 5.0 min) were selected for the culture sludge dissolved in distilled water [31]. The mass of polysaccharides was calculated using formula (1) based on the dry biomass of resorcinol sulfate (mg/g of d.m.).

2.4. NaOH heat treatment in the extraction of bound polysaccharides

A 7-day-old culture grown on Zarrouk medium was used for extraction. All chemical results are based on the actual dry weight of the microorganism. The initial pH of the cell suspension samples was 8, and the samples were further adjusted to pH = 9, 10, or 11 by adding 10 % NaOH solution. The obtained trace element suspension samples were

Table 1
Zarrouk nutrient medium composition.

Medium component	Concentration, g/L
$NaNO_3$	0.0–2.5
$K_2HPO_4 \times 3H_2O$	0.0–1.0
$Ti_2(SO_4)_3$	0.040
NaCl	0.0–1.0
MoO_3	0.015
$CaCl_2 \times 2H_2O$	0.04
H_3BO_3	2.86
$ZnSO_4 \times 7H_2O$	0.22
K_2SO_4	1.0
$NiSO_4 \times 7H_2O$	0.048
$FeSO_4 \times 7H_2O$	0.0249
$NaHCO_3$	0.0–16.8
$MnCl_2 \times 4H_2O$	1.81
$MgSO_4 \times 7H_2O$	0.2
$CuSO_4 \times 5H_2O$	0.08
NH_4VO_3	0.023
$Na_2WO_4 \times 2H_2O$	0.018
$K_2Cr_2(SO_4)_4 \times 24H_2O$	0.096
$Co(NO_3)_2 \times 6H_2O$	0.044

separated. Using an A24 thermostat (Millab, Moscow, Russia), pH values were determined at 25 °C, 45 °C, 65 °C, 85 °C, and 100 °C for 120, 180, and 240 min. The concentration of endopolysaccharide in the supernatant of the collected sample after centrifugation was determined every hour using anthrone sulfate. The mass of polysaccharides was then converted using the dry biomass (mg/g of d.m.) and anthrone sulfate method according to Eq. (1).

2.5. Purification of polysaccharides

Following isolation, microalgae polysaccharides were purified, including salt removal, in accordance with [32–34].

2.6. Content of uric acid

Uronic acid content was measured by the carbazole method. To reduce the effect of neutral lipids on the results on the microplate, 10 μL of sulfanilic acid in sulfuric acid (1 %) was added to 250 μL of suspension samples. The tubes were placed in a PE-4300 ice bath (Laboratoriya Pitera, St. Petersburg, Russia) and a solution of sodium tetraborate in sulfuric acid (0.1 %) was dripped into the pores. After that, the PE-4300 was placed in a boiling water bath and incubated for 6 min. A microplate with a mixture of sample and 50 μL of alcohol solution (0.1 %) was placed in the PE-4300 water bath for 10 min. After the tubes were cooled to room temperature, the optical density of the solution was measured at 525 nm. The content of uronic acids was determined from the calibration curve for galacturonic acid [35].

2.7. Content of neutral sugars

The resorcin sulfate method was used to measure the content of neutral sugars. Microbial sample (200 μL), resorcinol solution (200 μL) reconstituted in butanol (6 mg/L), and 75 % sulfuric acid were mixed. The tubes were shaken in a vortexer (DV-expert, Moscow, Russia) and heated in a PE-4300 water bath (90 °C, 30 min). Afterwards, the samples were cooled in the dark (30 min) on a PE-4300 ice bath. Using a standard curve for glucose solution, the content of neutral sugars was determined at an optical density of 480 nm.

All reagents used were purchased from Diem (Moscow, Russia) and were of analytical purity and highest quality.

2.8. Statistical analysis

The results of each experiment were presented as mean ± standard deviation. Three repetitions were performed in all experiments. Analysis of variance (ANOVA) was performed for the data using StatSoft 10.0 (StatSoft Inc., 2007, USA). Duncan criterion was applied to identify significant difference between the samples. Levene's test revealed equality of sample variance. If $p<0.05$, the differences between the mean values of the samples were considered significant. Polysaccharide yields were optimized by varying environmental conditions (nutrient medium components) [36]. Graphical representations were generated using Excel (Microsoft Corporation, 15.0, 2016, Redmond, Washington, DC, USA).

3. Results

3.1. Varying the composition of the nutrient medium

Initially, we optimized the composition of the nutrient media to maximize the accumulation of polysaccharides by *Nannochloropsis* spp. Limited nutrient supply is a widely used strategy for increasing polysaccharide accumulation in microalgae.

For this purpose, salts $NaNO_3$ (x_1), $K_2HPO_4 \times 3H_2O$ (x_2), $NaHCO_3$ (x_3), NaCl (x_4) were chosen as factors. The presence of these substances varied in the medium, while the remaining components were present at standard concentrations. As a result, the removal of sodium hydrogen carbonate and potassium hydrogen orthophosphate from the medium resulted in the highest yield of polysaccharides, with an EPS concentration of 0.63 mg/g d.w. The maximum increase in biomass was observed when potassium hydroorthophosphate and sodium chloride were removed from the medium; the biomass concentration was 1.02 mg/g d.w. (Fig. 1).

Taking into account the significance of the studied parameters, the regression equation was as follows:

$$y = 0.190 + 0.119x_1 - 0.220x_3 + 0.143x_4 \quad (2)$$

Our study demonstrated that microalgae biomass yield increased in the absence of NaCl in the nutrient medium, while EPS yield increased when K_2HPO_4 and $NaHCO_3$ were deficient.

3.2. Polysaccharide extraction

The actual weight of microalgae biomass was used in the studies. Analysis of the results of selecting the extraction module and the temperature regime for the extraction of exopolysaccharides from the culture liquid of *Nannochloris* sp. *Naumann* C-1509 (Table 2) indicates that the temperature and nature of the extractant are important physicochemical factors that determine the efficiency of polysaccharide extraction process. The removal of a small amount of the $NaHCO_3$ alkalizing solution did not significantly affect the growth of both microalgae and pH. The purification effect increased with increasing concentration of polysaccharides. The removal effect was 75.6 %−85.2 %.

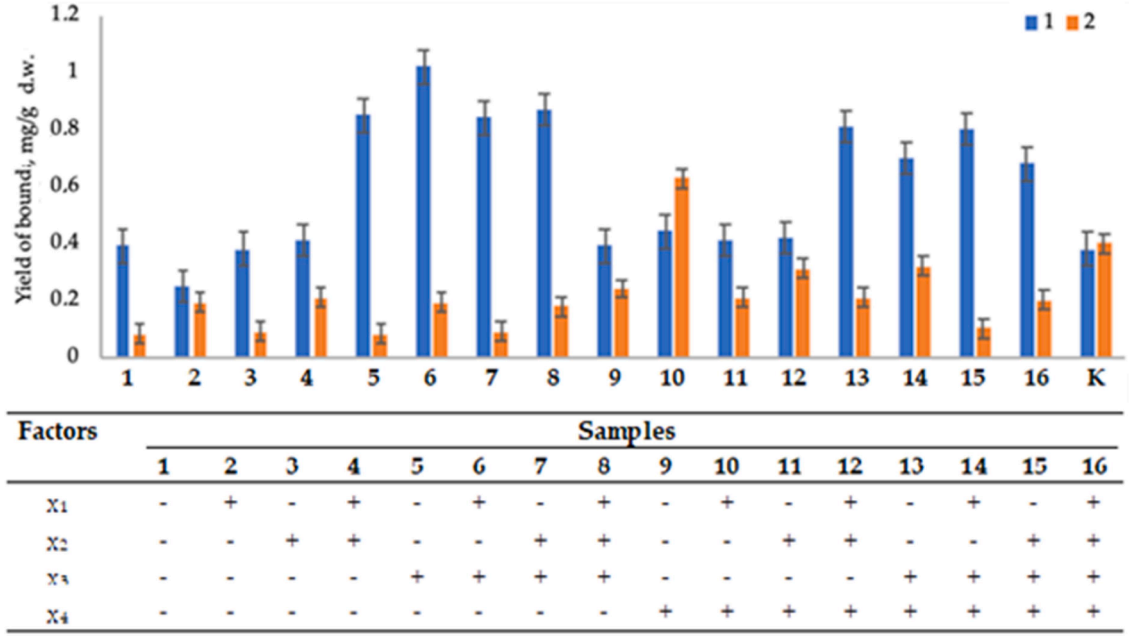
The greatest quantity of exopolysaccharides from the *Nannochloris* sp. *Naumann* microalgae was 304.51 mg/g dry biomass. The conditions of the extraction process were temperature minus 25 °C and hydromodule (ratio of culture liquid with ethanol) 1:2. The yield also decreased when the temperature droped to −35 °C. The process of crystallization of polysaccharides is accelerated by a decrease in their solubility at a given alcohol-to-sample ratio and temperature. Extraction with isopropanol at a module of 1:3 and a temperature of −25 °C produced a high yield of 259.93 mg/g of dry biomass. When the temperature is lowered by 10°, a similar quantitative yield can be seen. Butanol extraction reduced values by more than tenfold when compared to other extractants. It is also worth noting that when water-soluble extractants were added, a white suspension formed within the first few minutes of extraction. The lowest yield of polysaccharides was observed in NaOH extraction.

3.3. Ultrasonic extraction

Further we considered ultrasonic treatment of the culture liquid for isolation of endopolysaccharides. Endopolysaccharides and exopolysaccharides compose the total amount of polysaccharides. Microalgae cells, on the other hand, contain a significant amount of monosaccharides, which make up the carbohydrate component of microalgae. This study investigated alcohol extraction of exopolysaccharides and ultrasonic extraction of endopolysaccharides, the amount of which is different [36]. Results of polysaccharide extraction using ultrasonication from microalgae biomass is presented in Fig. 2.

The optimal conditions for ultrasonic treatment of *Nannochloris* sp. *Naumann* (C-1509) biomass samples were ultrasound power of 20–40 W and duration of 1–4 min. Under these conditions, the total amount of polysaccharides was isolated, however, it was found that with these parameters, the quantitative yield of exopolysaccharides reached 89.87 mg/g d.w. – the highest EPS count. Ultrasonic technology has been demonstrated to extract different polysaccharides. For example, Garcia-Vaquero isolated glucans in the amount of 219.5 mg/100 g of a.d.m. and fucose in the amount of 943.0 mg/100 g of a.d.m. using ultrasonic technology [37].

Under low temperature conditions, biomass growth was reduced and



Factors	Samples															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
x_1	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
x_2	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
x_3	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
x_4	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+

Fig. 1. EPS accumulation depending on the composition of the nutrient medium by the strain *Nannochloris* sp. *Naumann* (C-1509): 1 – Biomass; 2 – exopolysaccharides. x_1 - $NaNO_3$, x_2 - $K_2HPO_4 \times 3H_2O$, x_3 – $NaHCO_3$; x_4 – NaCl; K – Control.

Table 2
Conditions of the extraction process of exopolysaccharides from the *Nannochloris* sp. *Naumann* C−1509 culture liquid.

Extractant	Precipitation temperature, °C	Yield, mg/g dry weight 1:1*	1:2*	1:3*
Ethanol, 96 %	25	70.59 $^{a/a}$	85.35 $^{a/b}$	67.20 $^{a/a}$
	15	107.36 $^{b/a}$	184.04 $^{b/b}$	97.45 $^{b/c}$
	5	196.39 $^{c/a}$	231.83 $^{c/b}$	101.58 $^{b/c}$
	−15	209.93 $^{c/a}$	244.70 $^{c/b}$	189.18 $^{c/c}$
	−25	230.48 $^{d/a}$	**304.51** $^{d/b}$	204.15 $^{c/c}$
Isopropanol, 99 %	25	64.45 $^{a/a}$	68.94 $^{a/a}$	62.46 $^{a/a}$
	15	99.93 $^{b/a}$	106.23 $^{b/a}$	91.67 $^{b/a}$
	5	114.45 $^{b/a}$	222.22 $^{c/b}$	207.67 $^{c/b}$
	−15	197.51 $^{c/a}$	231.30 $^{c/b}$	215.06 $^{c/b}$
	−25	207.00 $^{c/a}$	268.04 $^{c/b}$	259.93 $^{d/b}$
Butanol, 99 %	25	58.67 $^{a/a}$	78.59 $^{a/b}$	84.76 $^{a/b}$
	15	88.98 $^{b/a}$	76.75 $^{a/b}$	127.99 $^{b/c}$
	5	96.27 $^{b/a}$	2.26 $^{b/b}$	121.22 $^{b/c}$
	−15	141.56 $^{c/a}$	4.51 $^{c/b}$	164.79 $^{c/c}$
	−25	175.10 $^{d/a}$	9.03 $^{d/b}$	144.47 $^{d/c}$
NaOH	25	48.33 $^{a/a}$	68.02 $^{a/b}$	71.18 $^{a/b}$
	15	64.71 $^{b/a}$	63.11 $^{a/a}$	96.91 $^{b/b}$
	5	73.48 $^{b/a}$	2.01 $^{b/b}$	113.02 $^{b/c}$
	−15	94.58 $^{c/a}$	3.93 $^{c/b}$	122.36 $^{b/c}$
	−25	108.26 $^{d/a}$	7.16 $^{d/b}$	124.77 $^{b/c}$

*Extraction module, (sample: alcohol). Column/line values followed by the same letter (a, b, c, d) are not significantly different ($p > 0.05$), assessed by Duncan's test. Data presented as a mean ($n = 3$).

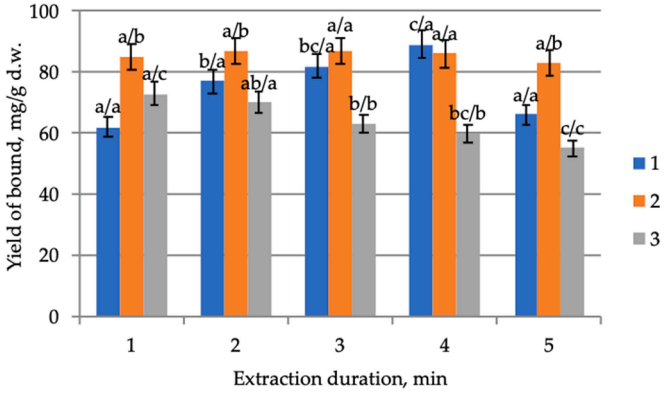


Fig. 2. Polysaccharide yield in extraction process using ultrasonic method: 1 – 20 W; 2 - 40 W; 3 - 60 W. Columns grouped by sonication power/duration, followed by the some letters are not significantly different ($p > 0.05$), assessed by post-hoc test (Duncan test). Data presented as a mean ± SD ($n = 3$).

a decrease in EPS yield was observed. Thus, the average specific EPS productivity of *Nannochloris* sp. Naumann decreased sharply. This is most likely due to the fact that when temperatures are low, the overall intensity of cell metabolism decreases. Increasing the cultivation temperature of *Nannochloris* sp. *Naumann* probably was a stressful condition and led to the activation of protective functions, and the release of specific carbohydrates into the environment.

Freeze-drying method was used for dehydration of polysaccharides isolated from microalgae samples. Temperature (−15 to −35 °C) and total duration of the drying cycle (8 h to 24 h) were varied to study the freeze drying of extract samples. The total duration of the process and the duration of the freeze drying stage were monitored by the temperature change in the polysaccharide concentrate layer. The least loss of exopolysaccharides was detected at −25 °C and a drying time of 16 h. For endopolysaccharides, these conditions were −35 °C and a duration of 16 h.

The largest total amount of polysaccharides (exo- and endopolysaccharides) was isolated at −25°C (Table 3). Neutral sugars in

Table 3
Dependence of polysaccharide content on drying temperature of microalgae extracts.

Indicator	Temperature, °C	Exopolysaccharides	Endopolysaccharides
Neutral sugars, mg/g PS	−15	24.5 ± 0.74[a]	10.0 ± 0.31[a]
	−25	31.0 ± 2.4[b]	27.3 ± 0.81[b]
	−35	40.0 ± 1.5[c]	24.6 ± 0.72[b]
Uronic acids, mg/g PS	−15	16.0 ± 0.28[d]	15.0 ± 0.28[c]
	−25	17.8 ± 0.28[d]	19.7 ± 0.31[d]
	−35	12.4 ± 0.27[e]	15.4 ± 0.27[c]

PS – polysaccharides. Column values followed by the same letter (a, b, c, d, e) are not significantly different ($p > 0.05$), assessed by Duncan's test. Data presented as a mean ($n = 3$).

polysaccharides were twice as abundant as uronic acids (Fig. 3).

The polysaccharides of microalgae contain polyuronides (acidic polysaccharides), which are insoluble in water and comprise uronic acids, which exhibit biologically active properties [38]. Therefore, the content of uronic acids in microalgae was studied. Neutral sugars are one of the major classes of chemicals of water-soluble polysaccharides of microalgae. They, like acidic sugars (uronic acids), determine the bioactive characteristics of polysaccharides and the possibility of their successful use in various applications (technological, research, etc.).

The concentrations of neutral sugars and uronic acids in the culture fluid were 40.0 ± 1.5 mg/g and 17.8 ± 0.28 mg/g of exopolysaccharides, and 27.3 ± 0.81 mg/g and 19.7 ± 0.31 mg/g of endopolysaccharides, respectively.

4. Discussion

Nutrients play an important role in the synthesis of amino acids, proteins, enzymes, vitamins, and pigments. Synthesis of various organic substances, including the accumulation of lipids and carbohydrates, is nitrogen deficiency dependent. The phosphorus content of the nutrient medium affects DNA and ATP synthesis. During microalgae growth, sulfur is required for basic cellular processes [39]. Phosphorus and sulfur deficiencies stimulate lipid and carbohydrate accumulation but inhibit protein synthesis [39]. Based on this information, we used $NaNO_3$, $K_2HPO_4 \times 3H_2O$, $NaHCO_3$; NaCl as the main components of the nutrient medium, the removal of which can cause cellular stress. Our study demonstrated the positive effect of salt deficiency in the nutrient medium on biomass production and polysaccharide yield.

Bacterial and plant cell metabolism and the rate of nutrient assimilation depend on temperature. Temperature is probably one of the key factors affecting microalgae cell growth, biomass accumulation, and the synthesis of secondary metabolites, particularly endopolysaccharides and exopolysaccharides [40]. As demonstrated in the study [41], the optimum temperature required for cultivation of microalgae ranges from 15 to 30 °C; damage or death of microalgae cells can occur outside this temperature range [35–37]. We have observed that polysaccharide release is increased at high temperatures (35 °C), but it is accompanied by cell death. Therefore, we considered the temperature optimum to be 20 °C, at which a positive dynamics of EPS and biomass concentrations was observed; their values were 1.37 g/l for EPS and 0.69 for the *Nannochloropsis* spp. biomass. According to the study [42], 20–25 °C was the ideal temperature for *Nannochloropsis* spp. to exhibit the highest concentration of polysaccharides.

Ultrasonic extraction is an efficient method for extracting various types of polysaccharides. Acoustic cavitation in ultrasonic extraction destroys bacterial and plant cells and reduces particle size, thereby promoting better contact between the extractant and the extracted substance and increasing the yield of the target product [43]. Because ultrasonic extraction uses less power, less solvent, is more efficient, and has a higher level of automation than chemical solvent extraction [44], it is preferred over chemical solvent extraction. The recommended

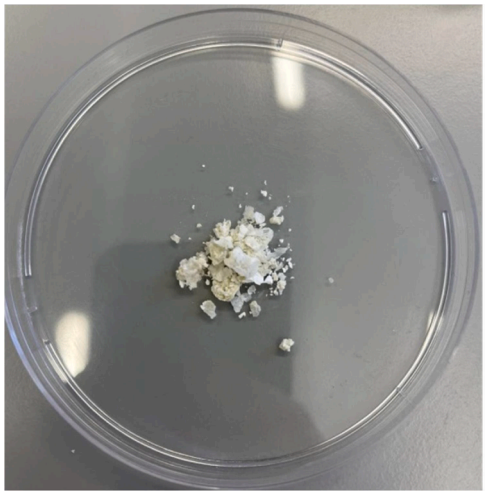


Fig. 3. A freeze-dried polysaccharide sample from the microalgae C-1509 *Nannochloris* sp. *Naumann.*

sonication conditions for *Nannochloropsis* spp. exopolysaccharides were the ultrasound power of 20 W and the duration of 4 min. The amount of bound polysaccharides decreased with increased processing time and power, most likely as a result of sample overheating and the breakdown of the polysaccharide structure.

Freeze drying is especially indicated for drying microalgae polysaccharides with high sensitivity to high temperature and oxygen, and can save high added value. In addition, the lyophilized polysaccharides are soluble [45,46]. However, freeze drying is expensive to set up and operate, particularly for industrial equipment, and drying time is lengthy (typically up to 12 h). We used this particular drying method, since it is recommended when it is necessary to preserve the biologically active functional groups of polysaccharides. Also, after analyzing the dried samples of polysaccharides, we found neutral sugars and uronic acids responsible for the biological activity of microalgae polysaccharides.

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CRediT authorship contribution statement

Conceptualization, Olga Babich and Stanislav Sukhikh; methodology, Aleksandr Tupitsyn, Ekaterina Budenkova, Egor Kashirskikh, Veronika Anokhova and Philippe Michaud; formal analysis, Svetlana Ivanova, Archana Tiwari, Aleksandr Vladimirov, Elena Nikolaeva, Ekaterina Budenkova, Veronika Anokhova and Stanislav Sukhikh; resources, Stanislav Sukhikh; writing—original draft preparation, Olga Babich, Ekaterina Budenkova and Veronika Anokhova; writing—review and editing, Svetlana Ivanova and Stanislav Sukhikh; project administration, Stanislav Sukhikh. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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