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Effect of cultivation conditions on polysaccharide synthesis by *Skeletonema pseudocostatum*

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Abstract:
Microalgae are a source of biologically active substances, e.g., polysaccharides. Their commercial potential attracts a lot of scientific attention. This research featured the effect of various nutrient media on the biomass of the psychrophilic microalga *Skeletonema pseudocostatum* and its ability to synthesize polysaccharides.

The Tamiya nutrient medium was used as the standard one. Its composition was improved to accelerate biomass cultivation. Optimization principles were based on the unconventional mathematical method for multidimensional modeling and involved the ANETR 21 software. The qualitative and quantitative assessment of microalgal polysaccharides relied on the anthrone sulfate method. The concentration of uronic acids was determined by the carbazole method while neutral sugars were studied by the resorcinol sulfate method in a microalgal suspension. The growth index of the *S. pseudocostatum* biomass was represented as a ratio of the maximal mass to the initial mass.

The maximal growth index was achieved by adding to the standard Tamiya medium: 10.00 g/cm^3 potassium nitrate ($MgSO_4 \times 7H_2O$), 2.50 g/cm^3 potassium dihydrogen phosphate, 1 cm^3 Fe^+ ethylenediaminetetraacetic acid (EDTA), and a mix of 4.29 g/cm^3 boric acid and 0.9 g/cm^3 manganese (II) chloride tetrahydrate ($MnCl_2 \times 4H_2O$) in an amount of 1.00 cm^3. The maximal polysaccharide biosynthesis was observed when the nutrient medium was modified as follows: 5.00 g/m^3 potassium nitrate, 3.75 g/cm^3 magnesium sulfate, 2.50 g/cm^3 potassium dihydrogen phosphate, 1 mm^3 solution of Fe^+ ethylenediaminetetraacetic acid, and solution of 1.0 g/cm^3 boric acid and 1.81 g/cm^3 $MnCl_2 \times 4H_2O$ (1.00 mm^3 each). The maximal accumulation of microalgal biomass was 2.88 ± 0.08 µg/100 mg dry solids; the maximal yield of polysaccharides was 3.16 ± 0.09 µg/100 mg dry solids. These results were obtained at 5°C.

The yield of polysaccharides by *S. pseudocostatum* depended on such cultivation parameters as temperature and pH. At cultivation temperatures of 0, 5, and 10°C, the yield of polysaccharides reached 2.13 ± 0.06, 3.16 ± 0.09, and 2.04 ± 0.06 µg/100 mg dry solids, respectively. The yield of exopolysaccharides represented by uronic acids and neutral sugars was 106.3 ± 3.1 mg/g and 806.6 ± 24.0 mg/g, respectively. In this research, polysaccharides synthesized by *S. pseudocostatum* demonstrated good prospects for the food industry and sustainable organic agriculture.

Keywords: Microalgae, *Skeletonema pseudocostatum*, polysaccharides, optimization parameters, nutrient medium

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INTRODUCTION

Microalgae are photosynthetic microorganisms that use light, energy, and carbon dioxide to produce biomass and bioactive compounds, e.g., polysaccharides [1]. They can convert solid waste from thermoelectric plants [2, 3] and other emissions, e.g., sulfur oxides, nitrogen oxides, or hydrocarbons, in order to use them as nutrients for growth. Microalgae are considered as excellent sources of functional and biologically-active nutrients to meet human nutritional needs [4]. *Porphyridium* sp.,

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Chlorella sp., *Spirulina* sp., and *Nostoc* sp. are the most well-studied sources in polysaccharide production [5].

Microalgae can grow under extremely unfavorable conditions due to their ability to synthesize polysaccharides [6]. However, some environmental and nutritional factors of microalgae technology may interfere with polysaccharide production [7]. For instance, salinity, nutrient deficiency, temperature, growth phase, as well as ions of magnesium, potassium, or calcium may affect polysaccharide synthesis [8]. Production conditions and infrastructure have to be optimized to cultivate microalgae with rapid cell growth rate, increased biomass, and high polysaccharide yield [9].

Polysaccharides obtained from microalgae consist mainly of galactose, xylose, and glucose, as well as some other sugars and glucuronic or galacturonic acid residues. Their composition depends on such factors as species, strain, and cultivation conditions. Acidic compounds, as well as sulfate and carboxyl groups, are also known to contribute to the anionic nature and, therefore, to the diverse biological activities of polysaccharides [10]. Exopolysaccharides are, perhaps, the most well-known microalgal polysaccharides due to their antioxidant, anti-inflammatory, antitumor, and antimicrobial properties. As a result, polysaccharides can be applied in different industries. Polysaccharides are necessary for the creation of new functional products, biologically active additives, pharmaceuticals, etc. [2, 5, 8, 10, 11].

Numerous publications on microalgal polysaccharides focus on their production, structure, and biological properties, which have direct application in various sectors. For example, Morais *et al*. [12] studied various aspects of obtaining exopolysaccharides from microalgae. Chanda *et al*. [13] reviewed microalgae polysaccharides as plant biostimulants. Moreira *et al*. [14] reported the use of microalgal exopolysaccharides in flocculation. These studies are crucial for commercial production of polysaccharides from microalgae. They explain the potential of microalgal polysaccharides for the food industry, animal feed, and agriculture.

Skeletonema pseudocostatum is a diatom microalga that can produce polysaccharides [15]. *Skeletonemae* are common phytoplankters and proliferate in coastal marine areas [16]. *S. pseudocostatum* is morphologically variable, genotypically diverse, and physiologically versatile. It lives in all seas and oceans, except Antarctica. The list of *Skeletonema* species includes *Skeletonema*, namely *S. ardens, S. dohrnii, S. grethae, S. japonicum, S. marinoi, S. menzelii, S. subsalsum,* and *S. tropicum* [17]. They differ in morphology and SSU/LSU rDNA sequence. In some cases, regional varieties may demonstrate environmentally-induced differences [17]. However, very little reliable information is available on the effect of the nutrient medium on the yield of exopolysaccharides.

Like others of its kind, *S. pseudocostatum* also possesses some exopolysaccharide prospects. This study featured the effect of various nutrient media on the biomass growth rate of *S. pseudocostatum*, as well as on its ability to synthesize polysaccharides.

In case of microalgae, the yield of exopolysaccharides depends on the modification of the standard nutrient medium and such cultivation variables as temperature, time, extractant, etc. Some studies [18, 19] confirmed that low nutrient content in a culture media encourages microalgae to produce more exopolysaccharides. Since *S. pseudocostatum* is a photosynthetic microorganism, an experiment with extremely low nutrient concentrations seems quite promising.

Exopolysaccharides develop as microalgae grow in mass. Their synthesis depends on particular conditions that occur as a result of nutrient depletion, high salinity, light pollution, etc. [20]. Depending on the photosynthetic microorganism, exopolysaccharides can be completely released into the medium or remain more or less bound to the cells.

Exopolysaccharides possess some unique rheological properties that make them promising gelling agents and thickeners. Microalgal exopolysaccharides may find a lot of prospective applications due to their unique properties and cheap production. They possess antibacterial, antioxidant, anti-inflammatory, antiparasitic, immunomodulatory, antitumor, and anticoagulant biological activities, which makes them a popular subject of *in-vitro* and *in-vivo* studies. To sum up, microalgal exopolysaccharides have a lot of advantages: a wide range of microalgal species to be used as raw material, unique structures, physicochemical properties, biological activity, etc. As a result, compounds like microalgal exopolysaccharides are attractive for many industries [21].

Exopolysaccharides are usually extracted from the culture medium by ethanol precipitation. Their yield depends on the length of the alcohol radical, as well as on the deposition temperature. Therefore, producers have to choose from ethanol, butanol, or isopropanol and apply optimal temperatures in the range from –30 to +30°C. Exopolysaccharides are thermally stable, which opens up opportunities for their use in the food, pharmaceutical, and cosmetic industries. Exopolysaccharides obtained using alcohols with different radical lengths differ in water solubility and water-holding capacity. As a result, they are quite predictable when used as hydrocolloids or stabilizers [22–25].

This research was the first of its kind to introduce the possibilities of producing polysaccharides by the psychrophilic microalga of *S. pseudocostatum* isolated from the Baltic Sea under various cultivation conditions. The study rationalized the composition of the nutrient medium and optimized the cultivating conditions with the maximal biomass and polysaccharide yield. The article also introduces data on the yield of uronic acids and neutral sugars.

STUDY OBJECTS AND METHODS

The research featured psychrophilic *Skeletonema pseudocostatum* from the Baltic Sea. The microalgae were cultivated at 0°C at night and +26°C during the day at a lighting intensity of 2,000–3,000 lux, 12-hour photoperiod, and an active acidity of 6.0–9.0. The cultivation

process took place in a refrigeration chamber on a laboratory orbital shaker Unimax 1010 (Heidolph) with a cooling module and a 267 mm box with a stirring speed of 120 rpm.

The first stage involved a standard Tamiya nutrient medium. The rationalized composition for accelerated biomass growth was determined using the unconventional method for constructing multidimensional models on a PC using the ANETR 21 software.

The growth index was represented as a ratio of the maximal yield to the mass registered at the cultivation onset.

Determining the rational composition of the nutrient medium. When using traditional methods, the final model is a polynomial that does not identify the effect of each individual factor on the final result. The ANETR 21 program [26] allows for mathematical multidimensional modelling and presupposes a special procedure that measures the effect of each factor, thus facilitating the optimization task.

We determined the composition of the nutrient medium for accelerated biomass cultivation under the following fixed conditions:

– the inoculum was 20% of the total suspension volume;
– the active acidity of the suspension was 6.0–9.0; and
– the photoperiod was 12 h and involved the LED SENAT Flora plant lamp (25 W).

The cell concentration was determined in a Goryaev chamber. The cultivation process consisted of two periods. During the first stage, microalgal biomass accumulated to ≥ 55–60 million cells/mL. During the second stage, we created stressful conditions to inhibit the proliferation of microalgae in the suspension and trigger the accumulation of polysaccharides in cells.

This method resulted in an analytical expression of the required multidimensional function. We also obtained graphs and analytical expressions of paired dependencies, a graph of changes in standard deviation (%), and deviations of the calculated values from the original value, all numbered and ranked in ascending order. The data about the model were generated into a form table.

To conduct a complete factorial experiment, we used a design matrix for nutrient media variants. We chose five arguments, i.e., components of the culture media.

The sequence of experiments correlated to the minimal costs on changing the level of the selected argument. The design matrix below involves m = 4–6; n = 5, where m is the number of arguments while n is the number of equations for each argument:

111111 122222 133333 144444 155555
215243 221435 234512 242351 253124
314325 323541 331254 345132 352413
413452 425314 432145 441523 454231
512534 524153 535421 543215 551342

The experiment followed the design matrix for 25 experiments.

If the required number of arguments (m) was ≤ 6, we removed the last columns of each minor rectangle from the matrix.

The matrix showed that at 6 = F(1,2,3,4,5), the factors that affected the growth index by optical density (%) ranked in order of effect priority as 3, 2, 5, 4, and 1.

Next, we performed a comparative analysis of the cultivation parameters on standard and optimized nutrient media. The analysis included the maximal density of the culture on the standard medium (P_{max}), as well as the initial (B_0) and maximal (B_{max}) densities of the culture on the optimized medium.

Quantitative analysis of polysaccharides. We determined the presence and quantity of polysaccharides using the anthrone sulfate method at each stage of parameter selection. According to the method, we added 150 μm of anthrone agent (0.1% recrystallized anthrone in concentrated sulfuric acid) to each microplate well (DV-expert, Moscow, Russia) with 50 μm of each sample. The plates spent 10 min at 4°C in a Pozis RK-102 S refrigerator (Diamond Elektrik, Moscow, Russia). After cooling, the samples were incubated in an A-24 thermostat (Millab, Moscow, Russia) at 70°C for 20 min and then cooled back to room temperature. The optical density was measured at 620 nm. The standard curve involved sucrose solutions [27].

Quantitative analysis of uronic acids. We applied the carbazole method to determine the level of uronic acids. To reduce the effect of neutral sugars, we added 10 μm^3 of sulfanilic acid in sulfuric acid (1%) to a 250 μm^3 suspension of microalgae. Then, we placed the tubes in a PE-4300 ice bath (PiterLab, St. Petersburg, Russia) and added 1.5 cm^3 of sodium tetraborate in sulfuric acid (0.1%) dropwise along the wall. After that, the samples underwent a boiling PE4300 water bath (PiterLab, St. Petersburg, Russia) for 6 min. Following this procedure, we added 50 μm^3 of carbazole solution in ethanol (0.1%) to the samples and again subjected them to the water bath for 10 min. The tubes were then cooled to room temperature to measure the optical density of the solutions at 525 nm. The concentration of uronic acids was determined using a calibration curve of galacturonic acid [28].

Quantitative analysis of neutral sugars. We used the resorcinol sulfate method to determine neutral sugars. We added 200 μm^3 of resorcinol recrystallized in butanol (6 mg/cm^3) and 1 mm^3 of 75% sulfuric acid to a 200 μm^3 of microalgae suspension. The tubes were shaken on a vortex (DV-expert, Moscow, Russia) and heated in the PE-4300 water bath at 90°C for 30 min. Subsequently, we cooled the samples in a PE-4300 ice bath in the dark for 30 min to measure the optical density at 480 nm. The concentration of neutral sugars was determined with a calibration curve of glucose solutions [29].

The reagents and chemicals were of analytical or higher grade (Diadema, Moscow, Russia).

Statistical analysis. All experiments and measurements were carried out in triplicates. The results were presented as a combination of mean and standard deviation (M ± SD). ANOVA was followed by Student's and Tukey's tests to identify statistically significant differences between different groups ($p < 0.05$). The statistical

analysis involved IBM SPSS Statistics 28.0.1 (IBM Corporation, USA) while the graphs were generated in Excel (Microsoft 300 Office, Microsoft Corporation, USA).

RESULTS AND DISCUSSION

The ANETR 21 software uses the Turbo Pascal language to process data bulks. The program makes it possible to introduce various restrictions depending on the nature of the phenomenon under investigation. Its paired dependencies include 15 equations. They describe curves that vary monotonically without extreme inflections, those with one inflection only, symmetrical/asymmetrical curves, and S-shaped curves.

The method is deterministic because the choice of the type of paired dependencies is limited for each argument, the passage of the paired dependency curve through a given point is mandatory, and a neutralization sequence is provided. The program includes some other restrictions that provide significant flexibility and versatility.

Table 1 shows calculations of the relative impact of the first three arguments on the required function.

The calculations in Table 1 provide the following data:

– Argument 3: 100.0 – 90.6 = 9.4%;
– Argument 2: 90.6 – 81.3 = 9.3%; and
– Argument 1: 81.3 – 76.2 = 5.1%.

The reliability of the models was assessed by several indicators, including the mean standard deviation of the calculated values from the original one (MSD) and from the mean intervals (RMS_{mean}) in absolute or relative units, %, the multiple correlation coefficient (*R*), and Fisher's criterion.

The design matrix data revealed the changes in the mean standard deviation (%). It was 6.2% while Fisher's coefficients were $F = 1.8086$, $F(001) = 2.697$, and $F(005) = 2.007$.

Table 2 represents the arguments and their values.

Two remaining factors 4 and 1 accounted for only 75.0 – 74.4 = 0.6% while the error caused by other unspecified factors was 6.2%.

As a result, factors 3, 2, and 5 proved significant for rationalizing the nutrient medium and physicochemical conditions for cultivating *Skeletonema pseudocostatum*.

When K_2HPO_4 (factor 3) grew from 0 to 3.62 g/cm^3, the biomass growth index increased from 69.3 to 90.6%, as in the equation $Y = 1/(0.0154 – 1.26\times10^{-6}\times X_3)$.

As the content of $MgSO_4\times2H_2O$ (factor 2) increased from 0 to 5.0 g/cm^3, the function initially grew from 61.0 to 96.0%, peaked at 2.50 g/cm^3, then dropped to 79.0%, as in $Y = –1.006 \times 10^{-7} \times X_2^2 + 4.18 \times 10^{-4} \times X_2 + 0.7104$.

As KNO_3 rose from 0 to 10.0 g/cm^3 (factor 1), the required function increased linearly from 66.0 to 84.5%, as in $Y = 0.8565 + 8.268 \times 10^{-4} \times X_6$.

In this experiment, the maximal growth index was observed at KH_2PO_4 – 8.4 g/cm^3, $MgSO_4\times7H_2O$ – 0.5 g/cm^3, and KNO_3 – 1.0 g/cm^3.

Table 2 made it possible to substantiate the effectiveness of a multifactorial experiment to rationalize the macrosalt composition of the nutrient medium. When *S. pseudocostatum* grew on a nutrient medium with an optimal macrosalt balance, its growth characteristics increased by a factor of 1.4, compared to the standard Tamiya medium.

Table 3 summarizes the experimental data.

Tables 4 compares the cultivation parameters of *S. pseudocostatum* on the standard and optimized nutrient media.

We registered a 1.5-time increase in biomass in the optimized medium, compared to the standard one.

Table 1 Model form

Argument	Equation	Coefficient			Mean standard deviation from the original, %	Multiple correlation coefficient	Mean standard from the mean intervals, %
		A	B	C			
3	7	1.539E-2	–1.263E-6	–	90.6	0.376	44.8
2	11	–1.006E-7	4.179E-4	7.104E-1	81.3	0.525	43.5
1	1	8.565E-1	8.268E-4	–	76.2	0.577	70.7
4	11	–9.704E-7	6.189E-4	9.688E-1	75.0	0.664	97.3
5	11	–5.483E-7	4.994E-4	9.398E-1	74.4	0.542	48.9

Table 2 Arguments (complete factorial experiment) and their values

No.	Argument, g/cm^3				
	1 KNO_3	2 K_2HPO_4	3 $MgSO_4\times2H_2O$	4 $(NH_2)_2CO$	5 $FeSO_4\times7H_2O$
1	0	0	0	0	0
2	0.09	0.009	0.009	0.9	0.0027
3	0.10	0.010	0.010	1.0	0.0030
4	0.11	0.011	0.011	1.1	0.0033
5	0.20	0.020	0.020	2.0	0.0060
Range	0–33.60	0–2.00	0–5.00	0–2.00	0–2.00

Table 3 Summary of experiments (n = 25) (complete factorial experiment)

Functions							
Values in design matrix						Experimental results	
						Growth index, %	Polysaccharides μg/ 100 mg
No.	1	2	3	4	5	6	7
1	0	0	0	0	0	0.757	0.01
2	0.09	0	0.0200	0.90	0.0030	1.442	2.08
3	0.10	0	0.0011	0.01	0.0027	0.980	2.02
4	0.11	0	0.0010	1.10	0.0060	1.105	2.41
5	0.20	0	0.0090	2.00	0.0030	1.143	2.02
6	0	0.009	0.0090	0.90	0.0027	0.676	2.12
7	0.09	0.009	0	1.10	0.0030	0.755	2.08
8	0.10	0.009	0.0100	2.00	0.0033	1.180	2.03
9	0.11	0.009	0.0200	1.00	0	1.187	2.01
10	0.20	0.009	0.0110	0	0.0060	1.210	2.01
11	0	0.010	0.0100	1.00	0.0030	0.708	2.02
12	0.09	0.010	0.0110	2.00	0	0.707	2.07
13	0.10	0.010	0	0.90	0.0060	0.790	1.02
14	0.11	0.010	0.0090	0	0.0033	1.302	2.02
15	0.20	0.010	0.0200	1.10	0.0027	1.341	2.52
16	0	0.011	0.0110	1.10	0.0033	0.686	2.19
17	0.09	0.011	0.0090	1.00	0.0060	0.790	2.10
18	0.10	0.011	0.0200	0	0.0030	1.302	2.63
19	0.11	0.011	0	2.00	0.0027	1.167	1.12
20	0.20	0.011	0.0100	0.90	0	1.076	2.09
21	0	0.020	0.0200	2.00	0.0060	0.757	1.43
22	0.09	0.020	0.0100	0	0.0027	0.797	1.87
23	0.10	0.020	0.0090	1.10	0	1.110	2.11
24	0.11	0.020	0.0110	0.90	0.0030	1.120	2.56
25	0.20	0.020	0	1.00	0.0033	1.208	2.15
Min	0	0	0	0	0	0.676	0.01
Max	0.20	0.020	0.0200	2.00	0.0060	1.341	2.63

Table 4 Comparative analysis of *Skeletonema pseudocostatum* cultivation parameters on standard medium

Strain	Maximal culture density on standard medium (P_{max}), %	Initial culture density on optimized medium (B_0), %	Maximal culture density on standard medium (B_{max}), %	Magnification coefficient
Skeletonema pseudocostatum	0.96	0.59	1.32	1.50

Therefore, the conditions improved in line with the complete factorial experiment proved to be optimal.

In this study, we rationalized the nutrient composition and optimized the physicochemical conditions for cultivating the microalgae. The maximal growth index was detected under the following nutrient composition: 10.00 g/L of potassium nitrate, 2.50 g/cm^3 magnesium sulfate, 2.50 g/cm^3 potassium dihydrogen phosphate, 1 mm^3 solution of Fe^+ EDTA (ethylenediaminetetraacetic acid), and 1.00 mm^3 solution of trace elements (4.29 g/cm^3 boric acid, 0.9 g/cm^3 $MnCl_2 \times 4H_2O$). The maximal polysaccharide biosynthesis in the antioxidant complex occurred under the following nutrient composition: 5.00 g/L potassium nitrate, 3.75 g/cm^3 magnesium sulfate, 2.50 g/cm^3 potassium dihydrogen phosphate, 1 mm^3 solution Fe^+ EDTA, and 1.00 mm^3 solution of trace elements (0 g/cm^3 boric acid, 1.81 g/cm^3 $MnCl_2 \times 4H_2O$).

S. pseudocostatum has received little scientific attention, most publications dating back to the 1970s and 1980s. However, there is another *Skeletonema Skeletonemataceae* microalga that found its way into research articles [30, 31]. Since both *S. pseudocostatum* and *S. costatum* belong to the same genus, we found it necessary to dwell upon the way *S. costatum* accumulates polysaccharides.

Myklestad [30] studied the effect of nitrogen:phosphorus ratio in the nutrient medium on the yield of *S. costatum*. The scientist varied the nitrogen:phosphorus ratio in six steps from 0.4 to 100 in batch cultures of the marine diatom of *S. costatum* (Greville) Cleve. The publication reports the effect of these changes on growth rate, nitrogen and phosphorus assimilation coefficient, yield of cellular and extracellular polysaccharides, protein, cellular phosphorus, and cell size in exponential and stationary growth phases.

At 10–100, the growth rate dropped from 1.7 to 1.1 divisions per day, and the rate of nitrate absorption from the nutrient medium also went down. At the lowest nitrogen:phosphorus ratios, the phosphorus content per cell was almost constant in both exponential and stationary phases and decreased as the ratio increased.

The fast-growing *S. costatum* cells proved to be poor polysaccharide producers whereas the slow-growing cells appeared to be much more prolific, as a result of media optimization.

As the nitrogen:phosphorus ratio grew, the ratio of cellular protein to polysaccharides decreased in the exponential phase and increased in the stationary phase. The *β*-1,3-glucan content was especially effective in boosting cellular polysaccharide synthesis. Myklestad [30] proposed the ratio of cellular polysaccharide to cellular phosphorus (15–675) as an indicator of the physiological state of marine diatoms. The study also casts light upon the potential ecological significance of *S. costatum* as a polysaccharide producer.

pH is an important factor for microalgal cultivation on optimized nutrient media. As a chemical property of the growing medium, pH affects the CO_2 hydrolysis and biodegradation of water-soluble sulfur-containing pollutants.

Low pH inhibits the growth of microalgae. However, CO_2 has a rather limited effect on pH while sulfur-containing ions can change pH significantly, thus reducing the biomass accumulation. In this research, we calibrated the pH values using a buffered medium.

Table 5 illustrates the effect of pH on *S. pseudocostatum* biomass growth rate at the initial and final stages of cultivation. The greatest increase in biomass and, therefore, polysaccharides was detected when the medium remained neutral throughout the entire cultivation process.

For *S. pseudocostatum*, the optimal ratio of initial and final pH values was 6.9/6.9. At this ratio, the biomass yield of *S. pseudocostatum* increased by 67.3 ± 0.14%.

Taraldsvik & Myklestad [31] grew *S. costatum* at different pH. They used a semi-continuous culture system that maintained a preset pH value. At pH 6.5–8.5, the growth rate was almost constant (2.4 divisions per day on average) and decreased at pH > 9. Organic carbon (C) production dropped from 5.3 mg/cm^3 to 2.0 at pH 6.5–8.5 and to 1.0 mg/cm^3 at pH 9.0 and 9.4. The content of *β*-1,3-linked glucan decreased from 7.1 mg/L at pH 6.5 to 0.2 mg/L at pH 9.4. The content of total organic carbon in the form of glucan decreased from 60 to 10%. At pH 8.0–9.0, the cellular concentration of amino acids varied from approximately 62 to 84 mM. At pH 9.4, the glutamine concentration sank below the detection limit. At pH 6.5–8.5, the content of extracellularly synthesized polysaccharides averaged as low as 3.7% of the total carbon but increased at pH 9.0 and 9.4. The down growth and biosynthesis of polysaccharides at pH > 9.0 might have been related to the decrease in the rate of some important biochemical reactions, as well as the changes in the properties of cell membranes in this pH range [31].

In our research, we defined the rational cultivation temperature after selecting the optimal pH. We varied it from 0 to 10°C. Table 6 summarizes the effect of temperature on the biomass growth and polysaccharide synthesis by *S. pseudocostatum*.

Table 6 shows it quite clearly that the largest yield of polysaccharides was detected at 5°C. At this temperature, exopolysaccharides entered the intercellular space most actively.

The biggest biomass accumulation (2.88 ± 0.08 g/cm^3) and polysaccharide yield (3.16 ± 0.09 μg/100 mg dry solids) were observed at 5°C. When we reduced the cultivation temperature to 0°C and increased it to 10°C, the biomass accumulation dropped to 1.92 ± 0.06 g/cm^3 and 2.13 ± 0.06 μg/100 mg dry solids, respectively, and the polysaccharide yield went as low as 1.71 ± 0.05 g/cm^3 and 2.04 ± 0.06 μg/100 mg dry solids, respectively.

Table 7 introduces the experimental data on the content of uronic acids and neutral sugars.

The optimized medium raised the yield of polysaccharides and uronic acids by a factor of 1.1. Neutral sugars demonstrated the greatest difference. In the optimized medium, they experienced a 1.48-time increase, probably, because the optimized environment provided the most favorable conditions for their accumulation.

Granum *et al*. [32] studied the cellular and extracellular synthesis of polysaccharides and amino acids by the marine diatom of *S. costatum* (Grev.) Cleve in different growth phases. They grew batch cultures on a 10/14 dark/light cycle in nitrogen-limited media at two different nutrient concentrations. The exponential

Table 5 Effect of cultivation medium pH on *Skeletonema pseudocostat*

Sample	Initial pH/final pH					
	5.0/4.9	6.2/6.2	6.9/6.9	7.5/7.8	8.0/7.9	8.3/7.9
Skeletonema pseudocostatum	55.20 ± 0.12	56.30 ± 0.14	67.30 ± 0.14	56.70 ± 0.13	58.10 ± 0.11	36.10 ± 0.08

Table 6 Effect of cultivation temperature on *Skeletonema pseudocostatum* growth and polysaccharide yield (antioxidant complex)

Sample	Biomass, g/cm^3			Polysaccharide yield, μg/100 mg dry solids		
	Cultivation temperature, °C					
	0	5	10	0	5	10
*Skeletonema pseudocostatu*m	1.92 ± 0.06	2.88 ± 0.08	1.71 ± 0.05	2.13 ± 0.06	3.16 ± 0.09	2.04 ± 0.06

Table 7 Effect of nutrient media on polysaccharide synthesis of *Skeletonema pseudocostatum*

Compound	Exopolysaccharides	
	Standard medium	Optimized medium
Polysaccharides, g/cm^3	2.88 ± 0.08	3.16 ± 0.09
Uronic acids, mg/g exopolysaccharides	96.90 ± 2.80	106.30 ± 3.10
Neutral sugars, mg/g exopolysaccharides	273.90 ± 8.10	406.60 ± 24.00

growth rate was 2.0 divisions per day. The growth rate was quite balanced, except for some significant changes in chemical composition. During the photophase, inorganic carbon and nitrogen were absorbed, and the number of elementary cells went down. At the end of the light phase of photosynthesis, the amount of *β*-1.3-glucan polysaccharide was 17% cellular organic carbon; at the end of the dark phase, it was 42%. The protein:glucan ratio was 2.3:0.7.

In the same study, cell wall polysaccharides accounted for 6–10% cellular organic carbon. The cellular pool of free amino acids rose from 8 at the end of the light phase to 22 at the end of the dark phase (% cellular organic nitrogen). Glutamine is the main amino acid in photosynthesis: its amount increased from 0.2 to 12 mmol per cell whereas the glutamine:glutamate ratio increased from 0.05 to 2. After depletion of nitrogen ions NO_3, the level of glucan increased rapidly within 3–4 days to settle down at the level of 75–80% cellular organic polysaccharide. On the contrary, the amount of cellular nitrogen decreased by 80%, and the amount of cell wall polysaccharides dropped by 35%. Consequently, the protein:glucan ratio dropped as low as < 0.1. The pool of cellular free amino acids decreased by 90% within 24 h of nitrogen depletion and continued to decrease steadily throughout the stationary phase. Glutamine decreased most rapidly and accounted for < 1% free amino acids in the stationary phase. Extracellular production accounted for 4% of total photosynthetic production during both exponential and stationary growth phases. However, the absolute release rate per cell was significantly higher in the exponential phase. In one case, a transient high release occurred during the transition phase, probably as a result of cell leakage. The extracellular production of healthy cells contained 33% of polysaccharides, 15% monosaccharides, and 5% free amino acids. The composition of extracellular amino acids differed from intracellular ones and experienced significant changes between the exponential and the stationary phases. To sum up, Granum *et al*. [32] reported the rapid response of the diatom *S. costatum* to ambient nitrogen and light conditions, which affected the carbohydrate and amino acid dynamics at the cellular level.

Biomass accumulation and polysaccharide synthesis by *Microchloropsis salina* reported by Ocaranza *et al*. [33] can serve as a comparison. *M. salina* is a source of various biomolecules, e.g., lipids and carbohydrates. Such polysaccharides as *β*-glucans are the most important synthesized carbohydrates to be used as antioxidants, antiseptics, and immunomodulators. Ocaranza *et al*. [33] assessed *M. salina* for its polysaccharide production potential under two different culture conditions: a high-density batch and a simulated high-density fed batch. The simulated high-density fed-batch culture improved the biomass growth. It was $8.00\times10^{-2} \pm 2.00\times10^{-3}$ g/cm^3 per 24 h; in the loading state, it was $-5.13\times10^{-2} \pm 4.00\times10^{-4}$ g/cm^3 per 24 h. The same dependence occurred for *β*-glucans: their volumetric production reached $5.96\times10^{-3} \pm 2.00\times10^{-4}$ g of product/cm^3 per 24 h vs. $4.10\times10^{-3} \pm 2.00\times10^{-4}$ g product/cm^3 per 24 h under batch conditions. These data establish the basic conditions for optimizing and boosting polysaccharide biosynthesis and biomass growth. This new raw material source of *β*-glucans can be applied in the production of nutraceuticals for people and animals. To sum up, Ocaranza *et al*. [33] described the accumulation of polysaccharides by *M. salina* in standard and modified media.

S. pseudocostatum is a promising alternative for polysaccharide synthesis in the sense that it can mitigate the effects of industrial pollution [34]. *S. pseudocostatum* is known to grow faster than any land plant, and it requires no fertile soil. Therefore, it does not compete with food production. Its carbon fixation efficiency is 10–50 times greater than that of any plant. Microalgae help reduce greenhouse gases by sequestering carbon dioxide from industrial processes. *S. pseudocostatum* can grow in fresh, sea, subsaline, and waste water [35]. Microalgal polysaccharides have important rheological and biological properties for food and sustainable agriculture applications.

CONCLUSION

The improved nutrient medium and pH ratio at the onset and end of cultivation (6.9/6.9) yielded 2.13 ± 0.06, 3.16 ± 0.09, and 2.04 ± 0.06 μg polysaccharides per 100 mg dry solids at 0, 5, and 10°C, respectively. When we reduced the cultivation temperature to 0°C or brought it up to 10°C, the *Skeletonema pseudocostatum* biomass and the polysaccharide yield decreased. Similar results occurred when we lowered or raised the ratio of pH values at the two cultivation stages. The polysaccharide synthesis in the optimized medium demonstrated a 1.3-time increase. The polysaccharide yield in the optimized medium was 1.1 times greater than in the standard medium. The uronic acid synthesis demonstrated the same 1.1-time increase in the optimized medium. The neutral sugars showed the largest 1.48-fold difference between the standard and the optimized media. The optimized nutrient medium provided the most favorable conditions for the accumulation of neutral sugars.

It had no hydrodynamic factors that would inhibit the cell growth, which allowed it to boost the polysaccharide synthesis.

Polysaccharides obtained from *S. pseudocostatum* can be used to design new types of foods, e.g., healthy bakery products, special nutrition for sports and weight correction, age-inhibiting products, new dietary supplements, pharmaceuticals, etc.

CONTRIBUTION

V.F. Dolganyuk – 25%, E.V. Kashirskikh – 10%, S.A. Sukhikh – 20%, O.E. Kremleva – 10%, E.V. Ulrikh – 10%, D.I. Malkov – 10%, O.O. Babich – 25%.

CONFLICT OF INTEREST

The authors declared no conflict of interest related to this publication.

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