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Application of Extracellular Polymeric Substances during Cultivation of Microalgae Biomass

Paulina Rusanowska, Marcin Zieliński *, Magda Dudek and Marcin Dębowski

Department of Environmental Engineering, Faculty of Geoengineering, University of Warmia and Mazury in Olsztyn, 10-720 Olsztyn, Poland; paulina.jaranowska@uwm.edu.pl (P.R.); magda.dudek@uwm.edu.pl (M.D.); marcin.debowski@uwm.edu.pl (M.D.)

* Correspondence: marcin.zielinski@uwm.edu.pl

Featured Application: The biomass of microalgae/cyanobacteria selected in this study had different characteristics and applications and included *Chlorella* sp. (bio-oil production), *Tetraselmis subcordiformis* (bio-hydrogen production), and *Arthrospira platensis* (phycocyanin production). A critical, well-diagnosed, and proven problem of microalgae/cyanobacteria cultivation on an industrial scale is the lack of a simple and effective technology for separating the obtained biomass from the culture medium. The study aimed to solve the problem of biomass harvesting using extracellular polymeric substances extracted from activated sludge.

Abstract: Extracellular polymeric substances (EPS) produced by microorganisms contain polymers that are used for the bioflocculation of microalgae; however, these polymers are also organic compounds that might be used as carbon sources. The study analyzed two strategies for the introduction of EPS for *Tetraselmis subcordiformis*, *Chlorella* sp., and *Arthrospira platensis* biomass harvesting. In the first variant, EPS in the dose of 100 mg TOC/g were added to the photobioreactor every other day from the beginning of the cultivation, while in the second variant, EPS in the two doses of 100 mg TOC/g and 300 mg TOC/g were only added at the end of cultivation. In the first variant, the results proved that microalgae/cyanobacteria can use the EPS as external carbon sources. The cultures were characterized by a faster increase in biomass concentration, which contained less chlorophyll. However, the EPS content did not change. In the second variant, the addition of EPS did not affect the EPS content and the sedimentation of the *Chlorella* sp. biomass. The biomass of *T. subcordiformis* was characterized by a much better sedimentation coefficient. The greatest differences were observed in the *A. platensis* culture: the biomass concentration increased from 1.2 ± 0.2 g/L to 1.9 ± 0.2 g/L, EPS content increased by 16%, and sedimentation efficiency increased to 72%.

Keywords: biomass harvesting; EPS; bioflocculation; mixotrophic growth

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1. Introduction

EPS secreted by microorganisms are complex compositions of biopolymers with a high molecular weight, such as polysaccharides, proteins, humic substances, nucleic acids, and lipids [1]. EPS serve to shield cells from environmental stresses, such as dehydration, external heavy metals, and toxic compounds, and helps to maintain matrix stability [2]. Polymers included in EPS retain stable matrix structures and form a network for cells to interact with each other and mediate their adhesion to surfaces.

One of the possible applications of extracellular polymers is the bioflocculation of microalgae biomass. Technologies using microalgae biomass are solutions with significant potential that can be applied in broadly understood field of environmental engineering. Research carried out so far has proved that it is possible to use systems for the intensive cultivation of microalgae biomass in wastewater and leachate treatment, waste and sewage

sludge management, carbon dioxide biosequestration, or flue gas purification. An important problem of microalgae cultivation on an industrial scale is the lack of simple and effective technology for the separation of the biomass from the medium.

Centrifugation involves using a rotational force that accelerates the mobility and detachment of particles by different densities of the particle and the medium. This process, because of its high efficiency and simplicity, is the most commonly employed technology for industrial microalgae harvesting [3]. However, it is not recommended for certain species due to shear stress. The disc stack centrifuges enable the separation of microalgae of small sizes (3–30 mm) with low cell densities. However, the high capital costs and considerable energy requirements make this technology unprofitable [4].

Filtration is the separation of solid and liquid via a membrane with pores that allow microalgae medium to pass through while retaining cells [5]. Membrane fouling is the biggest issue with using this technology, and it usually requires various improvement strategies to increase its efficiency [6].

Flocculation is a process that requires the presence of flocculating agents inducing microalgal cells to aggregate, which facilitates easier and faster sedimentation. Flocculants can be inorganic, organic, or have biological origin. Currently, research has focused on developing integrated systems for simultaneous microalgae culture and cell harvesting, such as the co-culture of microalgae and fungi, the co-culture of microalgae and bacteria, using microalgae biofilms, etc. These systems show a high density of microalgae cells and efficient cell separation from the medium realized in the same reactor [7]. However, most of these approaches are still in the early stages of development, and future research should focus on improving and optimizing these integrated culture and harvesting systems.

Bioflocculation is initiated by extracellular polymers (EPS) produced by well-settling microorganisms (microalgae, bacteria, fungi, or yeast). The advantage of bioflocculation is its non-toxic nature and potential as an economic method for the concentration of microalgae biomass. EPS added for bioflocculation determine the settling properties and dewatering of flocs. The bioflocculant produced by *Solibacilus silbestris* was used to concentrate microalgae *Nannochloropsis oceanica* [8]. The bioflocculant did not require the addition of other coagulants and was not toxic to microalgae cells. In addition, the research presented the possibility of its recovery after applying filtration and evaporation. The results indicate that the bioflocculation efficiency of the recovered bioflocculant dropped by only 3%, which significantly reduces the costs of biomass concentration. However, these results are only attractive for small-scale processes. In addition, the presented approach is connected to the necessity of purchasing a specific bacterial species, its cultivation, and the isolation of the bioflocculant in a separate reactor, which may pose technological difficulties and significantly increase the costs of biomass production.

There are many studies of the use of different substances to improve the sedimentation properties of microalgae. However, according to the authors' knowledge, EPS extracted from activated sludge from wastewater treatment plants have never been used for this purpose. However, as EPS are rich in organic carbon, they can also play a role as extracellular energy and carbon sinks. They are an abundant source of structurally and compositionally diverse biopolymers. Therefore, EPS are important carbon sources for different organisms in the food chain.

The separation method for microorganisms' EPS can, in turn, influence the compositions, contents, structure, properties, and functions of EPS [9,10]. Commonly applied methods, e.g., high-temperature or alkaline environments, could markedly change the properties and compositions of the extracted EPS. The cation-exchange resin method was selected as the most effective for the extraction of EPS, which prevents external forces from causing chemical contamination and changing the characteristics and properties of EPS [11,12].

The presence of both proteins and polysaccharides in the extracted EPS could stimulate the aggregation of cells or production of EPS by microalgae, which can contribute to the better sedimentation properties of microalgae. Thus, the aim of this study was to assess the

possibility of the utilization of extracellular polymeric substances (EPS) produced by the biomass of bacteria used in wastewater treatment (activated sludge) to densify the biomass of selected species of microalgae and cyanobacteria. The research analyzed the impact of isolated EPS on the cultivation of microalgae *Chlorella* sp., *Tetraselims subcordiformis*, and cyanobacteria *Arthrospira platensis*.

2. Materials and Methods

2.1. EPS Origin

EPS were extracted from activated sludge using an aerobic reactor with a volume of 20 L, which was fed with model municipal wastewater with a high load of organic compounds. The basic technological parameters of the bioreactor operation were as follows: the concentration of activated sludge in the chamber was 5200 mg/L, load of organic compounds was 0.7 kg COD/(m^3·d), and oxygen content was 2 mg/L. During the operation of the aerobic bioreactor, the sedimentation time of the activated sludge was successively shortened in the range from 15 to 5 min. Then, EPS were extracted and characterized. The EPS content was expressed as mg of total organic carbon (TOC)/g biomass. The extracted EPS were characterized by 429 ± 34 mg TOC/g VS, 310 ± 18 mg of proteins (PN)/g biomass, and 61 ± 9 mg of carbohydrates (PS)/g biomass.

2.2. EPS Extraction

EPS were isolated using ion exchange resins [13]. In brief, EPS extraction was performed using a Dowex 50 × 8, Na+ Form cation-exchange resin (Fluka). The ratio of the rinsed cation-exchange resin to VS of biomass was 35 g to 0.5 g. The prepared samples were stirred at 750 rpm for 4 h in the dark at 4 °C. The blank sample was prepared with PBS buffer and cation-exchange resin. Then, the samples were centrifuged at 10,000 rpm for 1 min and again centrifuged at 10,000 rpm for 10 min in new clean tube. Afterwards, the samples were filtered using the membrane filter (0.22 µm pore size). The total quantity of extracted EPS was measured using an Elementar High TOC analyzer (Shimadzu TOC-L, Kyoto, Japan).

2.3. Microalgae Cultivation

Microalgae cultures were carried out in tubular reactors with an active volume of 2.5 L, equipped with a lighting and air mixing system. Based on the experience gained during the implementation of previous research, the cultures of *Chlorella* sp. and *A. platensis* were illuminated in a continuous mode, while *T. subcordiformis* was illuminated in the mode of 14 h of light/10 h of darkness. The cultures were illuminated by cool-white light at 700 lx using a T8 Sylvania Gro-Lux 18 W fluorescent tube. The temperature in the photobioreactors was kept at 22 ± 1.0 °C for *Chlorella* sp. and *T. subcordiformis*, and *A. platensis* was kept at 30 ± 2.0 °C. The composition of the *T. subcordiformis* medium was as described elsewhere [14], and the *A. platensis* medium was as described by [15], while for the cultivation of *Chlorella* sp., a modified BBM medium (bold basal medium) was used, the composition of which was described in [16]. The air introduced into the photobioreactors from the bottom ensured the mixing of the cultures and was a carrier of carbon dioxide. Each experiment was performed in triplicate. The taxonomic biomass changes were controlled at microscope total magnifications of 100× or 400× using the algae analyzer BBE (Moldaenke, Schwentinental, Germany).

2.4. Bioflocculation

This study analyzed two variants used to introduce EPS into the photobioreactor for the cultivation of microalgae/cyanobacteria. In the first variant, EPS were introduced into the photobioreactor every other day from the beginning of the culture (V1), while in the second variant, EPS were only introduced at the end of microalgae/cyanobacteria growth (on 7th day). In the first variant, EPS in the amount of 100 mg TOC/g of microalgae were

introduced into the culture. In the second variant, EPS in the amounts of 100 mg TOC/g (V2) and 300 mg TOC/g (V3) of microalgae were introduced into the culture (Figure 1).

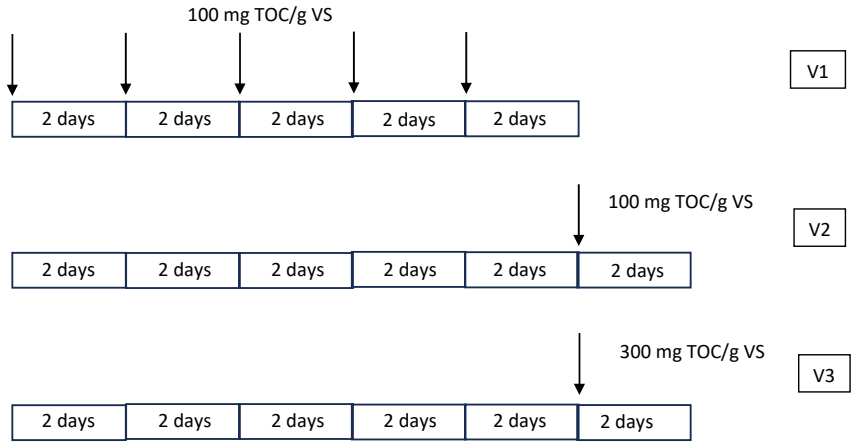


Figure 1. The scheme of the experiment.

The biomass characteristics included the determination of the contents of total solids (TS), volatile solids (VS), and chlorophyll. The medium characteristics included the determination of total nitrogen (Ntot), phosphorus (Ptot), and organic compounds (COD). The EPS characteristics included the determination of polysaccharides and proteins.

2.5. Analytical Methods

The TS and VS concentrations were assessed via a gravimetric method. The samples of nutrients and organic analyses in the medium were first centrifuged via a laboratory centrifuge MPW-251 (Donserv, Warsaw, Poland) with a rotational speed of 5000 rpm for 10 min. The COD, Ntot, and Ptot concentrations were measured via LCK cuvette tests (Hach-Lange, Berlin, Germany) using the DR 5000 spectrophotometer with the HT 200 s mineralizer (Hach-Lange, Berlin, Germany). EPS were isolated from the biomass using ion exchange resins collected at the end of the experiments. The total quantity of extracted EPS was measured using an Elementar High TOC analyzer (Shimadzu TOC-L, Kyoto, Japan). Lowry's method [17] was used to measure the amount of protein, relative to a standard curve created using bovine serum albumin (BSA). Anthrone method [18] was used to measure the amount of carbohydrates, relative to a standard curve created using glucose. The Assay kits were used for the specific measurement of monosaccharides, disaccharides, and trisaccharides (arabinose, fructose, glucose, gluconic acid, fucose, glucosamine, glucuronic acid, galactouronic acid, lactose, galactose, sucrose, maltose, raffinose, rhamnose, trehalose, and xylose) (Megazyme Ltd., Bray, Ireland). Only the content of carbohydrates higher than 1 mg/g VS was presented in the results. The content of chlorophyll a was measured via extraction with acetone and spectrophotometrically determined at 661.7 nm with a calibration curve using chlorophyll a as standard (Sigma-Aldrich, Burlington, MA, USA) [19,20]. The sedimentation efficiency was measured in cylinders with a capacity of 100 cm^3 after 30 min. The cylinders had a reference scale to record the results in millimeters.

After testing for the homogeneity of variance via Levene's test, the significance of the differences between the variants was tested via Tukey's HSD test. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

The study tested different strategies used for the possible application of EPS for microalgae/cyanobacteria biomass. In strategy V1, the biomass was characterized by lower chlorophyll content compared to the control cultivation (Table 1). In this mixotrophic cultivation (V1), the chlorophyll content was significantly reduced by 72.5%, 71.4%, and 50% in the biomass of *T. subcordiformis*, *Chlorella* sp., and *A. platensis*, respectively ($p < 0.05$).

The introduction of EPS during the cultivation in V1 caused microalgae/cyanobacteria to use polymers as an external carbon source.

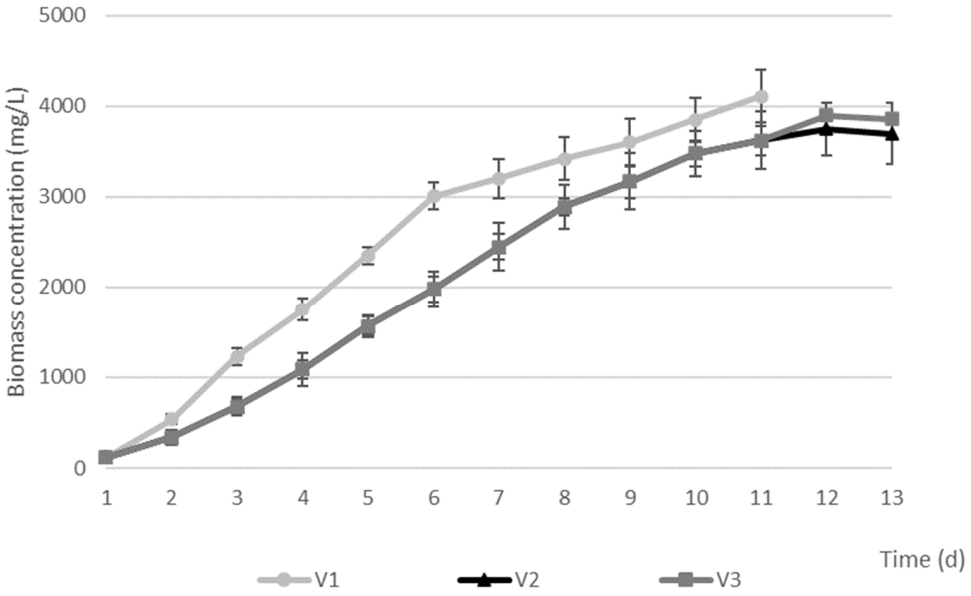
Table 1. Biomass concentrations, chlorophyll content, EPS content, and sedimentation efficiency obtained in the variants of experiments during the cultivation of *T. subcordiformis*, *Chlorella* sp., and *A. platensis*.

	Variant of Experiment	Biomass Concentration (mg/L)	Chlorophyll Content (mg/g VS)	EPS Content (mg TOC/g VS)	Sedimentation Efficiency (%)
Tetraselmis subcordiformis	C	3621 ± 174	40 ± 11	95 ± 11	40
	V1	4109 ± 160	11 ± 4	97 ± 7	42
	V2	3698 ± 102	32 ± 9	97 ± 25	55
	V3	3856 ± 111	28 ± 12	97 ± 10	66
Chlorella sp.	C	3920 ± 289	28 ± 8	60 ± 20	32
	V1	4210 ± 320	8 ± 6	61 ± 9	31
	V2	4290 ± 255	13 ± 5	62 ± 12	34
	V3	4321 ± 262	10 ± 2	61 ± 19	36
Arthrospira platensis	C	1213 ± 182	16 ± 4	81 ± 6	49
	V1	1512 ± 191	8 ± 4	83 ± 9	54
	V2	1760 ± 195	11 ± 6	91 ± 12	68
	V3	1912 ± 150	9 ± 4	96 ± 2	72

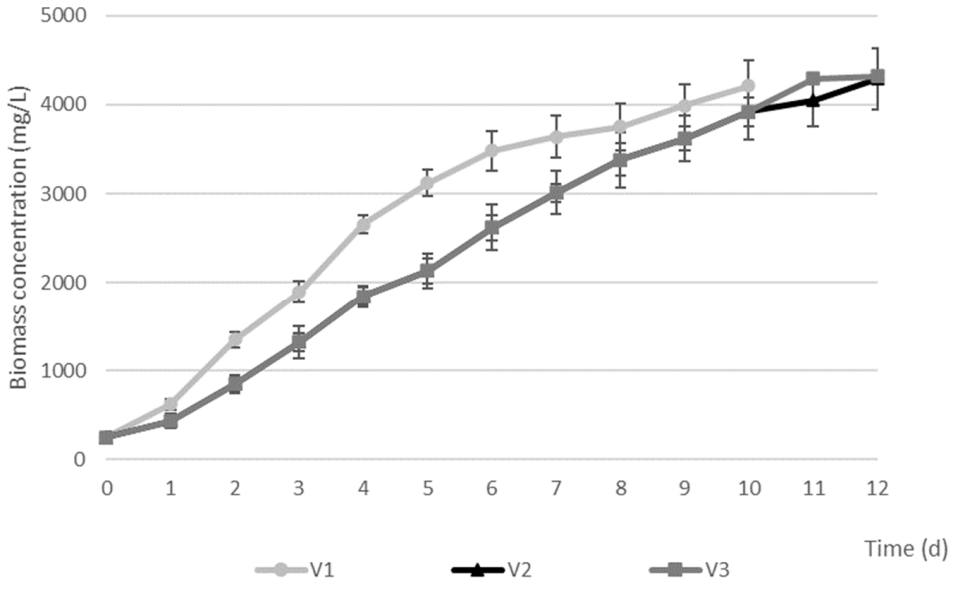
Photoautotrophic microorganisms use light as a source of energy for CO_2 assimilation and store light energy in the chemical bonds of organic molecules. Some of these microorganisms also have a capacity for heterotrophic growth or mixing heterotrophic and photoautotrophic growth in metabolic traits called photomixotrophic traits. The strategy for changing metabolic traits is used for fast acclimation to the environmental conditions of aquatic microorganisms. The environmental conditions that could modulate the metabolic traits and, therefore, growth of microorganisms include light intensity, temperature, and concentrations of different forms of carbon. The ability of microorganisms to change metabolic traits is poorly described in the literature and has remained rather undefined. It was found that among microalgae species, *Chlorella* sp. is capable of mixotrophic growth [21,22]. The studies proved that higher biomass concentrations were observed under mixotrophic growth than under only photoautotrophic or heterotrophic growth. The presence of organic and inorganic carbon sources during mixotrophic conditions results in combining photosynthetic activity with high intracellular inorganic carbon concentrations created by glucose breakdown and the release of CO_2, despite the direct utilization of glucose-derived carbon skeletons for growth [22,23].

During the proteomic analysis under different metabolic conditions in *Chlorella* sp. cultivation, it was observed that after feeding the photoautotrophic culture with glucose to start the mixotrophic metabolism, the chlorophyll content in biomass decreased immediately, and this decline lasted 8 h. During the next 24 h, the transition from the mixotrophic conditions to the photoautotrophic conditions occurred [22]. The present study showed slightly different results: the chlorophyll content in biomass did not increase in the two days after the addition of organic carbon (EPS). These differences might be the result of different sources and concentrations of organic carbon being added to cultivation. EPS consist of polysaccharides, proteins, nucleic acids, and lipids, which could exert a longer effect on cell metabolism than simple compounds, such as glucose. The presence of organic carbon sources inhibits the synthesis of pigments at the start of mixotrophy, causing the lower chlorophyll content [24]. Moreover, the reduction in the abundance of proteins from the thylakoid membranes and the downregulation of photosynthesis-related genes were reported under mixotrophic conditions [25]. Similar observations of lower chlorophyll content in the cultures with organic carbon addition were observed during cultivation of *Arthrospira platensis* [26] and *Tetraselmis subcordiformis* [14,27].

The biomass growth in the control and until 10th day of microalgae/cyanobacteria cultivation in V2 and V3 was the same since on the 10th day after EPS were added to the control cultivation. The biomass concentration during strategy V1 was higher than during the control for all tested microalgae/cyanobacteria. During V1, the biomass concentration of *T. subcordiformis* and *Chlorella* sp. was similar, and the growth rate was around 600 mg/(L·d) in the first 5 days of cultivation and around 100 mg/(L·d) in the next 5 days (Figure 2). The *A. platensis* growth rate was steady during cultivation, being around 150 mg/(L·d).

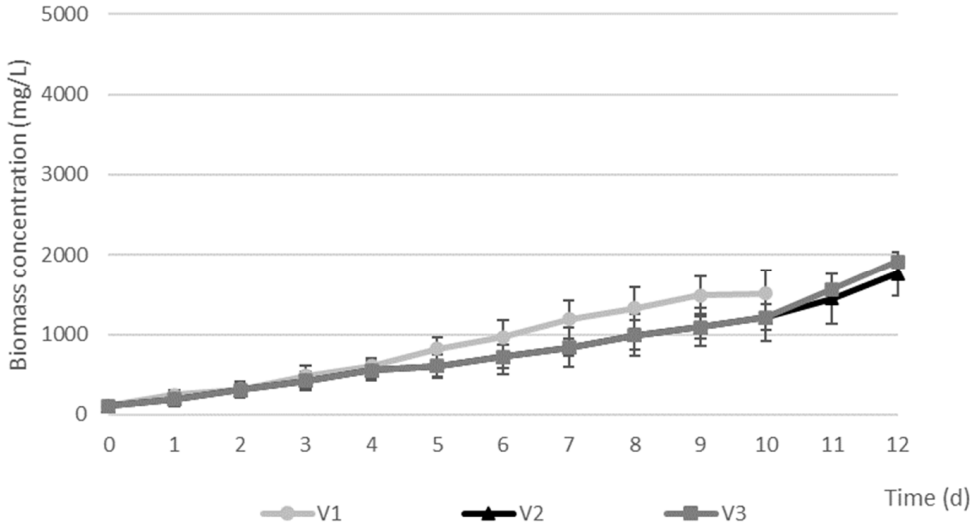


(**A**)



(**B**)

Figure 2. *Cont.*



(**C**)

Figure 2. Microalgae/cyanobacteria growth during different strategies of EPS addition to cultivation: (**A**) biomass concentration of *T. subcordiformis*; (**B**) biomass concentration of *Chlorella* sp.; (**C**) biomass concentration of *A. platensis*.

Microalgae growth generally can be divided into six phases during batch cultivation: the lag phase, exponential phase, linear phase, declining growth phase, stationary phase, and death phase. In the lag phase, the growth of the cell is delayed due to the presence of non-viable cells or adaptation to new environmental conditions (the addition of organic carbon). In the present study, the lag phase was not observed for all tested strategies. Similar observations for *Chlorella vulgaris* were noted during transcriptomic studies of the transition of photoautotrophic to mixotrophic metabolism, in which biomass after the addition of glucose started an exponential phase [22]. Additionally, the studies of terrestrial cyanobacteria cells under heterotrophic and mixotrophic conditions did not show a lag phase [28]. The lag phase for *C. sorokiniana* during mixotrophic cultivation with different volatile fatty acids lasted less than 1 day, which can also be the explanation for observations in the present study because the measurements were taken once a day [29]. The above-mentioned explanations support the statement of mixotrophic metabolism observed during EPS addition in strategy V1.

The mixotrophic cultivation did not increase the sedimentation efficiency of *T. subcordiformis* and *Chlorella* sp. biomass (Table 1). The biomass of *A. platensis* was characterized by a 5% better efficiency of sedimentation after mixotrophic cultivation than autotrophic cultivation. The introduction of EPS at the end of cultivation (V2 and V3) also lowered chlorophyll content in biomass; however, the reduction was not significant (Table 1). In V3, the chlorophyll content was lower than in V2. The higher concentrations of source of organic carbon available for biomass, the greater the reduction in the chlorophyll content in biomass. In V2 and V3, the chlorophyll content was reduced by about 25%, 60%, and 38% compared to autotrophic cultivation in the biomass of *T. subcordiformis*, *Chlorella* sp., and *A. platensis*, respectively. The sedimentation efficiency was not improved in *Chlorella* sp. when EPS was introduced at the end of cultivation, independently of the EPS concentration. However, the sedimentation efficiency of *T. subcordiformis* and *A. platensis* were improved. In the case of *T. subcordiformis*, the better sedimentation properties were not connected to higher EPS content, because in all variants of the experiments, the EPS content was similar. However, in *A. platensis* biomass, the EPS content increased.

The EPS content in the control biomass of microalgae/cyanobacteria and the biomass from the microalgae/cyanobacteria culture to which EPS of the activated sludge were added during cultivation did not differ. The use of mixotrophic conditions did not affect

the improvement in the sedimentation properties and EPS content. It is well known that increased carbon availability during cultivation will increase the biomass concentration and decrease the chlorophyll content [30]. Moreover, mixotrophic cultivation also often has a positive effect on the "production capacity" of microalgal cells, e.g., *Chlorella* sp. often has more oil in the cells, while *A. platensis* has more phycocyanin in the cells [31].

In this study, in the EPS samples, the protein content, sugar content, and carbohydrate composition were determined (Table 2). In the EPS extracted from *T. subcordiformis* and *Chlorella* sp., the protein content was similar in variants of experiments. However, in the EPS extracted from *A. platensis*, the protein content increased. The positively charged amino groups in proteins can neutralize the negative charges of polysaccharides, uronic acid, and carboxylic acid in DNA, phosphate groups, etc.; thus, proteins might effectively change the surface properties of the cell, inducing the formation of aggregates [13,32,33]. The higher content of proteins in *A. platensis* could improve the sedimentation properties of biomass.

Table 2. EPS composition obtained in the variants of experiments during the cultivation of *T. subcordiformis*, *Chlorella* sp., and *A. platensis*.

	Variant of Experiment	Protein Content (mg/g VS)	Sugar Content (mg/g VS)	Carbohydrates			
				Glucose (mg/g VS)	Mannose (mg/g VS)	Rhamnose (mg/g VS)	Galactose (mg/g VS)
Tetraselmis subcordiformis	C	27.4 ± 6.7	21.5 ± 4.5	15.2 ± 5.4	-	-	1.3 ± 0.5
	V1	28 ± 4.8	25.3 ± 5.6	21 ± 1.1	-	-	1.9 ± 0.4
	V2	27.9 ± 3.5	32 ± 3	24.3 ± 2.7	-	-	1.9 ± 1.5
	V3	28.3 ± 9.2	41 ± 5	22.5 ± 3.2	-	-	2.1 ± 0.8
Chlorella sp.	C	32.9 ± 4.7	41.1 ± 4	17.8 ± 3.2	12.9 ± 5.2	-	1.1 ± 0.2
	V1	33.1 ± 8.8	50.3 ± 8.2	23.8 ± 2.5	19.4 ± 3.8	-	1.7 ± 0.1
	V2	33.7 ± 5.3	53.7 ± 9.1	24.1 ± 5.3	20.1 ± 2.7	-	1.7 ± 0.5
	V3	35.6 ± 4.9	56.3 ± 7.7	23.9 ± 4.5	21.1 ± 3.2	-	2.2 ± 1.1
Arthrospira platensis	C	45 ± 10.3	37.8 ± 6.4	11.4 ± 1.2	-	25.1 ± 3.2	10.9 ± 1.4
	V1	59.1 ± 9.1	42.8 ± 9.8	10.5 ± 5	-	26.7 ± 1.9	8.6 ± 3.1
	V2	57 ± 13.2	45.2 ± 8.1	11.9 ± 2.1	-	29.6 ± 0.5	9.1 ± 4
	V3	60.1 ± 11	48 ± 10.1	12.4 ± 2.9	-	30.4 ± 3	9.0 ± 3.2

In the EPS extracted from *T. subcordiformis*, sugar content has significantly increased in the variants of experiments where EPS were added at the end of microalgae growth (V2 and V3) compared to autotrophic cultivation (Table 2). Among the measured carbohydrates, the highest concentration was glucose, the concentration of which also increased in V2 and V3; however, it was not a significant increase. The higher content of sugars in the EPS might be caused by some other exopolysaccharide that also influenced the better sedimentation of *T. subcordiformis* (Table 1). Exopolysaccharide produced by *Bacillus subtilis* was used for harvesting *Nannochloropsis oculata* and *Botryococcus braunii* biomass [34]. The authors obtained the best flocculation with the dose of 5% v/v of exopolysaccharide and pointed out that harvesting efficiency and flocculant concentration are not linearly dependent. Polysaccharides were also connected to the formation of algal-bacterial bioflocs. The sedimentation properties of bioflocs were better with a higher content of polysaccharides [35]. Similarly, polysaccharides were responsible for cell capture and aggregation during the rapid biogranulation of the microalgae *Ankistrodesmus falcatus* var. *acicularis* [36]. The polysaccharide derived from *T. subcordiformis* should be further investigated via extraction and analyses to determine its influence on the sedimentation properties.

In the EPS extracted from *Chlorella* sp., the sugar content increased in the variants to which EPS were added at the end of microalgae growth (V2 and V3) compared to autotrophic cultivation (Table 2). The main components of carbohydrates in the EPS extracted from *Chlorella* sp. were glucose and mannose. Despite the increase in sugar content, the EPS content and sedimentation efficiency did not improve. Based on the results of the EPS content and composition and sedimentation properties of *T. subcordiformis*

and *Chlorella* sp., it might be concluded that similar flocculants induce the production of different exopolysaccharides with different properties.

The applied strategy and flocculant did not improve the sedimentation properties of *Chlorella* sp. Similar observations were made by [37]. The authors tested the settling properties of two species of microalgae, namely *Scenedesmus* sp. and *Chlorella vulgaris*, with and without cultures of the bacterium *Burkholderia cepacia* and bacterial exudates. *C. vulgaris*' settling properties were not improved by applying flocculants at any growth stage. In contrast, glutathione significantly increased the EPS content in *Chlorella pyrenoidosa* and the settling properties above 72% [38]. The biomass of *Chlorella vulgaris* could even have 94% harvesting efficiency when the culture was contaminated with bacteria [39]. The stress conditions that could include bacteria presence, nitrogen depletion, etc., could induce the autoflocculation of microalgal cells and improve sedimentation [40]. The stress and autoflocculation might also be the results of changes in cell surface properties. It was proved that the cell surface charge of *Chlorella* sp. is different during the growth phases of biomass. These changes are related to the formation of a protective layer at the cell wall during late cultivation stages [41]. However, in the present study, the EPS added to the microalgae culture probably did not cause stress conditions for the cells of *Chlorella* sp. and was only a good source of external carbon for changing the metabolism for mixotrophic cultivation.

In the EPS extracted from *A. platensis*, the sugar content increased. The carbohydrates detected in all variants of experiments were glucose, rhamnose, and galactose. The addition of EPS at the end of the growth phase (V2 and V3) only increased the content of rhamnose in the EPS extracted from *A. platensis*. In the comparison of the mixotrophic and heterotrophic cultivation of *A. platensis* with whey as an external carbon source, the growth rate and protein and lipid contents were higher under heterotrophic cultivation; however, chlorophyll-a and total carbohydrates were higher in the mixotrophic culture [30]. In the present study, the cultures of *A. platensis* with the addition of EPS at the end of the growth phase were also characterized by higher protein content that, together with the carbohydrates, increased the sedimentation properties of this microalgae.

The differences in the bioflocculation of different species might be attributed to differences in cell size and shape, culture density, and EPS produced [20]. *Chlorella* sp. has the smallest cells, with a diameter range from 2 to 10 μm. The other important factor seems to be type of EPS produced by microalgae. As mentioned above, well sedimentation properties of *A. platensis* were connected to exopolysaccharide extracted by microalgae.

4. Conclusions

The study can be summarized based on the following conclusions:

- Extracellular polymeric substances from bacteria can be used as an external carbon source by microalgae/cyanobacteria;
- Extracellular polymeric substances can be used for harvesting *Tetraselmis subcordiformis* and *Arthrospira platensis*;
- The sugar content in *Tetraselmis subcordiformis* biomass was responsible for the higher sedimentation coefficient.

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