

Original Research

Applicability of water from the Bay of Gdańsk as a growth medium for mixotrophic culture of *Platymonas subcordiformis*

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Abstract

P. subcordiformis is a potentially promising species with commercial, environmental and technological viability for industrial applications. The great potential of these microalgae lies in their fast biomass growth, pollution resistance, and compatibility with different culture media. This study aimed to determine the efficiency of *P. subcordiformis* biomass production in a medium prepared with water from the Bay of Gdańsk. The tested medium supported high biomass growth rates which reached $317.58 \pm 42.31 \text{ mg}_{VS}/\text{dm}^3 \cdot \text{d}$ in the best-performing variant, with a final concentration of $3493.3 \pm 465.4 \text{ mg}_{VS}/\text{dm}^3 \cdot \text{d}$. In the autotrophic culture, nitrogen and phosphorus removal exceeded 98%. Amending the mixotrophic culture with glucose did not affect *P. subcordiformis* concentrations. However, it did significantly limit the demand for nutrients in the biomass and reduced chlorophyll a production in the cells.

Keywords: Microalgae; *Platymonas subcordiformis*; Mixotrophic culture; Natural water; Growth medium

1. Introduction

Pushing forward the development and widespread take-up of environmentally-friendly, efficient and cost-effective biomass production technologies represents an ongoing challenge to scientists, as well as a priority issue for operators of energy systems [1]. There have been numerous attempts to demonstrate that typical land energy crops can be used for this purpose [2,3]. However, the use of such plants requires appropriate agrotechnical procedures, marginal/degraded soils, natural fertilizers, as well as species of energy crops that must be carefully selected, fast-growing and resistant to environmental conditions [4].

However, this prevailing view has been contested by some opinions and publications. Life cycle analyses (LCA) have shown that mismanagement of traditional energy crop reserves may actually exacerbate greenhouse gas emissions [5]. There is an increasingly common view that intensive use of farmland for growing biofuel crops may lead to decreased global food supply and a significant positive pressure on food prices [6]. Such risks and limitations have also been acknowledged by European Union (EU) legislators. Reports on ILUC (*Indirect Land Use Change Impacts*), commissioned by the European Commission, call into question the environmental viability of biofuels produced from grain, root/tuber crops or oil crops [7]. As such, the feasibility of using food sources for biofuel production is an increasingly common thread in discussions at the EU level. It is generally assumed that these goals are to be achieved, e.g., by harnessing advanced biofuels made from

alternative feedstocks [8].

Therefore, there is a real need to seek alternative sources of biomass, ones which would be both commercially and environmentally viable as a source of energy. Algae possess very high photosynthetic efficiency, can rapidly build biomass, are resistant to various contaminants and can be sited on land that is unsuitable for other purposes. Given these considerations, algae may represent a viable substitute for traditional energy crops. Algae also appear on the European list of feedstocks which, when used for biofuel production, can be double-counted for the purposes of determining biofuel energy content [9,10]. Microalgae biomass is also a rich source of carbohydrates, proteins, fatty acids, enzymes and fibers. In addition, algae are rich in vitamins and minerals, including vitamins A, C, B₁, B₂, B₆, as well as niacin, iodine, potassium, iron, magnesium and calcium. Cyanobacteria and green algae are an excellent source of protein and saturated fatty acids, phycocyanin and beta-carotene [11]. A dynamically developing direction is the production of astaxanthin, which is one of the main xanthophyll dyes [12]. Among the microalgae capable of producing astaxanthin is *Haematococcus pluvialis* [13]. Microalgae also have the ability to synthesize such fatty acids like, eicosapentaenoic acid (EPA 20: 5 ω3) [14] and docosahexaenoic acid (DHA 22: 6 ω3) [15,16]. The positive effect of DHA and other Omega-3 fatty acids on the cardiovascular system has been proven in many *in vivo* and *in vitro* experiments and clinical trials [17].



A key factor, which in many cases determines the cost-effectiveness of energy production from algal biomass, is the choice of technology used to grow and separate the organisms [18]. Algae can be cultivated using various methods, beginning from strictly monitored methods in technologically advanced designs, to less predictable methods based on open systems [19]. Apart from the technology used to produce the biomass, the type and, most importantly – cost of the growth medium used also play a huge role [20]. Therefore, there have been attempts to identify relatively cheap methods which would harness various types of sewage, leachate, industrial effluent or waste water [21]. Use of natural water as growth medium is a novel area of research that very few papers have dealt with so far [22]. The vast majority of the data available pertain to producing microalgal biomass on media prepared from distilled water and chemical reagents that provide optimal conditions for microalgal growth [23,24].

One promising species, which may be commercially, environmentally and technologically viable, is *P. subcordiformis*. Some literature reports show that these microorganisms can be a source of value-added substances such as sugars, proteins or fats, given optimal culture conditions [25]. The great potential of *P. subcordiformis* algae lies in their fast biomass growth, resistance to various types of pollution, high adaptability, and compatibility with different culture media of various physico-chemical parameters [26]. There is also a fast-expanding body of research on using *P. subcordiformis* planctonic algae by switching their metabolism towards hydrogen production [27,28].

This study aimed to determine the efficiency of biomass production on a mixotrophic *P. subcordiformis* culture. The medium was prepared with Bay of Gdańsk water as the base.

2. Materials and methods

2.1 Experimental design

The study was separated into two series with the growth medium as the differentiating factor. Series 1 (S1) utilized deionized water, whereas series 2 (S2) used water sourced from the Bay of Gdańsk. Each of the series was divided into two variants. In variant 1 (V1), the medium was supplemented exclusively with microelements required for optimal growth of *P. subcordiformis*. In the second variant (V2), the medium was amended with glucose as an external carbon source to expedite the growth of the microalgal population.

2.2 Microalgal biomass

The study used halophilous microalgae of the species *P. subcordiformis*, sourced from the UTEX collection (The University of Texas at Austin). The *P. subcordiformis* inoculum was transferred to the agar medium in a 20 cm³ tube. In the first phase, the microalgae were grown in sterilized Falcon tubes with an active volume of 50 cm³. After 11

days of culture, the biological specimens were transferred to DURAN bioreactors with a capacity of 1.0 dm³ to initiate the second phase of growth. Air was supplied into the reactor via Mistral 200 peristaltic pumps with a flow rate of 200 dm³/h. The PBR was incubated in a Binder MB1 chamber (with a programmable controller) at 25 ± 1.0 °C and intermittently exposed to 5 klux white light. The experiment followed a 14 h light/10 h dark regime, in accordance with literature indications [29]. The bioreactors were pasteurized using a Tuttnauer 2840 EL-D autoclave at 121 °C for 15 minutes. In the first phase, the microalgae were grown in a medium prepared according to literature [29,30] (Table 1). The resultant biomass was used in the exact experiment.

Table 1. Composition of the synthetic medium used to grow *P. subcordiformis*.

Constituent	Value	Unit
FeCl ₃	1.30	mg/dm ³
MnCl ₂	0.36	mg/dm ³
H ₃ BO ₃	33.60	mg/dm ³
EDTA	45.00	mg/dm ³
NaH ₂ PO ₄	20.00	mg/dm ³
NaNO ₃	100.00	mg/dm ³
ZnCl ₂	0.21	mg/dm ³
CoCl ₂	0.20	mg/dm ³
(NH ₄) ₄ Mo ₇ O ₂₄	0.09	mg/dm ³
CuSO ₄	0.20	mg/dm ³
VB12	0.10	μg/dm ³
VB1	1.00	μg/dm ³
Parameter	Value	Unit
Salinity	30–33	ppt
pH	8.00–8.20	–

The profile of the microalgal biomass is presented in Table 2. Initial microalgal biomass with dry organic matter levels (VS) and chlorophyll levels during the exact experiment were kept at 200 mg_{VS}/dm³ and 0.1 μg/dm³. In all of the variants, *P. subcordiformis* was cultured over a period of 11 days. Samples for analysis were taken every 48 h.

2.3 Growth media

The study used growth media with different profiles depending on the experimental series. In series 1 (control), the medium was primarily composed of deionized water supplemented with trace element-rich sea salt (Aqua Medic Reef Salt) to ensure that salinity levels were close to 30 ppt.

Series 2, on the other hand, utilized water collected from May to September from the Bay of Gdańsk. Before being used to grow *P. subcordiformis*, it was first filtered through Eurochem medium-grade Ø 12.5 qualitative filter paper, then sterilized in a Tuttnauer 2840 EL-D autoclave at

Table 2. Profile of the *P. subcordiformis* biomass used in the experiment.

Parameter	Unit	Value
Mineral solids (MS)	% TS	73.97 ± 6.23
Volatile solids (VS)	% TS	26.03 ± 6.23
Total nitrogen (TN)	mg/g VS	5.32 ± 1.42
Total organic carbon (TOC)	mg/g VS	50.74 ± 15.34
Total carbon (TC)	mg/g VS	54.34 ± 11.27
Protein	mg/g VS	33.25 ± 4.26
Lipids	% TS	18.0 ± 2.0
C:N ratio	–	10.21 ± 1.52
pH	–	7.89 ± 1.02

121 °C for 15 minutes. In all of the experimental series of the first variant, the medium base was supplemented with optimum concentrations of nutrients, as shown in Table 1. In variant 2, the system was amended with 10 g/dm³ crystal glucose (AR quality). Detailed profiles of the Bay of Gdańsk water and the growth media are presented in Table 3.

2.4 Experimental set-up

The exact experiment was conducted in a New Brunswick BioFlo 115 bioreactor with an active volume of 2.0 dm³ (temperature 25 ± 1 °C, with intermittent exposure to 5 klux white light under a 14 h light/10 h dark regime). The FBR was connected to a Mistral 200 air pump with a flow rate of 200 dm³/h. The reactor contents were continuously mixed at 150 rpm using agitator blades. The biomass was separated using a Whatman vacuum membrane filter with a 50 mm filter insert composed of mixed cellulose esters (porosity 8.0 µm). A pump fitted in the bioreactor sub-unit was used to transfer the medium and the biomass to the filtration system consisting of a filter inserted into a Whatman MBS 1 filtration system, enabling separation of the algal biomass from the growth medium via a Mobil 20 vacuum pump.

2.4 Analytical methods

2.4.1 Qualitative and quantitative analysis of the biomass

The taxonomic analysis of the algal biomass was done using an MF 346 biological microscope with an Optech 3MP camera. The study included a qualitative analysis of the microalgal biomass used as inoculum in the reactors. Chlorophyll was determined spectrophotometrically after extraction with 90% acetone. The TS, VS, and MS were measured gravimetrically. Total protein content was estimated by multiplying TN by a protein conversion factor of 6.25. Lipids were determined using the Soxhlet method with a Buchi extraction apparatus. The pH determination procedure was as follows: 10 g of the homogenized air-dried sample was weighed out to a 100 mL beaker; 50 mL of distilled water was added; the mixture was then stirred; and the pH of the resultant sample was finally measured with a

calibrated apparatus. Total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) were determined using a Shimadzu TOC analyzer.

2.4.2 Profile of the growth medium

The water collected from the Bay of Gdańsk for growing *P. subcordiformis* was tested for salinity, dissolved oxygen, and pH using a Hach Lange HQ 440D multi-parameter meter and an Aqua Medic Marine Control Digital salinity meter. Total nitrogen, ammonia nitrogen, total phosphorus, orthophosphates, sulphates, chlorine compounds, iron compounds, and COD were determined using Hach Lange cuvette tests and an UV/VIS DR 5000 spectrophotometer. The same methodology was applied to monitor the levels of essential nutrients in the culture, i.e., TN and TP. Light intensity was measured with a HANNA HI 97500 luxometer. Glucose concentration in the growth medium was monitored using a YSI 2700 Select analyzer.

2.4 Statistical analysis

Each experimental variant was conducted in triplicate. The statistical analysis was conducted and determination coefficient R² was computed using STATISTICA 13.1 PL (Statistica 13.1 software, StatSoft Inc., Tulsa, OK, USA, 2016). One-way analysis of variance (ANOVA) was used to determine significant differences between the groups. Significant differences between the variables were determined via Tukey's HSD test. Results were considered significant at $p = 0.5$.

3. Results

The biomass concentration in S1V1 was 3203.3 ± 35.1 mg_{VS}/dm³ (Fig. 1), with chlorophyll levels at 3686.7 ± 320.8 µg/dm³ (Fig. 2). Biomass growth rate (r) reached 291.21 ± 3.19 mg_{VS}/dm³·d. After 11 days of culture, nitrogen and phosphorus were almost completely spent, with levels at 0.61 ± 0.37 mg TN/dm³ and 0.04 ± 0.01 mg TP/dm³, respectively (Figs. 3,4). The average specific nutrient take-up for biomass growth was 7.00 ± 0.45 mg TN/g_{VS} and 1.69 ± 0.09 mg TP/g_{VS} (Figs. 5,6). In S1V2, amendment of the medium with glucose did not significantly ($p = 0.05$) affect the resulting *P. subcordiformis* population (Table 4). The VS in the PBR was 3348.3 ± 89.9 mg_{VS}/dm³ at $r = 304.39 ± 8.17$ mg_{VS}/dm³·d (Fig. 1). On the other hand, significant differences were observed with regard to chlorophyll levels in the system (Table 4). Chlorophyll concentration was 1986.7 ± 151.7 µg/dm³, with mean r at 180.61 ± 13.80 µg/dm³·d (Fig. 2). These values are nearly 50% lower than those noted in S1V1 (Fig. 2). Nutrients were only partially utilized by the growing *P. subcordiformis* (Figs. 5,6). Phosphorus levels at the end of the culture period were 0.66 ± 0.09 mg TP/dm³ (Fig. 4). Nitrogen take-up was approximately 75.70 ± 0.60% (Fig. 7), which translates to N levels of 5.11 ± 0.13 mg TN/dm³ (Fig. 3).

The r value in S2V1 was 317.58 ± 42.31 mg_{VS}/dm³·d,

Table 3. Profile of the raw water sourced from the Bay of Gdańsk.

Parameter	Unit	Bay of Gdańsk	Series 1 (S1)		Series 2 (S2)	
			Variant 1 (V1)	Variant 2 (V2)	Variant 1 (V1)	Variant 2 (V2)
COD	mg O ₂ /dm ³	38.13 ± 3.96	55.5 ± 2.01	11033 ± 57.73	59.97 ± 0.25	11133 ± 152
Ammonia nitrogen	mg NH ₄ -N/dm ³	0.04 ± 0.01	0.04 ± 0.01	0.039 ± 0.01	0.04 ± 0.01	0.038 ± 0.01
Total nitrogen	mg TN/dm ³	0.63 ± 0.12	21.13 ± 0.63	20.79 ± 0.56	21.85 ± 1.05	21.41 ± 0.26
Orthophosphates	mg PO ₄ ³⁻ /dm ³	0.18 ± 0.05	1.85 ± 0.24	13.53 ± 0.25	2.95 ± 0.79	16.6 ± 0.25
Total phosphorus	mg TP/dm ³	0.06 ± 0.01	5.00 ± 0.20	5.04 ± 0.14	5.05 ± 0.31	5.27 ± 0.03
Sulphates	mg SO ₄ ²⁻ /dm ³	413 ± 5.03	525 ± 1.00	521 ± 6.11	538 ± 2.52	545 ± 2.57
Chlorine (I)	mg Cl ⁻ /dm ³	2353 ± 5.77	7510 ± 10	7318 ± 2.89	7411 ± 59.5	7705 ± 32.88
Chlorine (II)	mg Cl ⁻ /dm ³	333 ± 2.08	16128 ± 55.2	16113 ± 15.26	2380 ± 156	18240 ± 20
Iron (II)	mg Fe ²⁺ /dm ³	0.09 ± 0.01	0.09 ± 0.01	0.103 ± 0.06	0.107 ± 0.01	0.113 ± 0.02
Iron (III)	mg Fe ³⁺ /dm ³	0.14 ± 0.02	0.09 ± 0.01	0.102 ± 0.07	0.087 ± 0.01	0.076 ± 0.01
pH	–	7.63 ± 0.19	8.09 ± 0.12	8.08 ± 0.09	8.04 ± 0.15	8.12 ± 0.10
Salinity	ppt	–	30 ± 0.7	30 ± 0.9	30 ± 0.3	31 ± 0.8

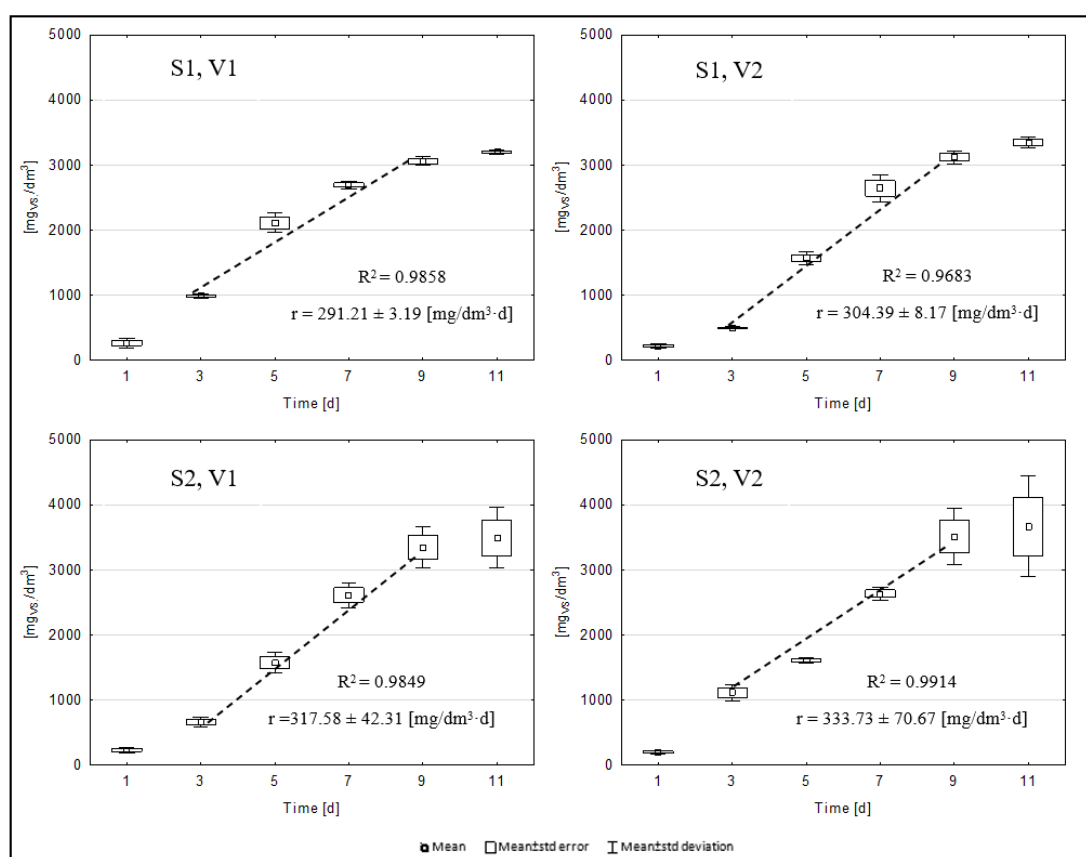


Fig. 1. Trends in VS concentration in the growth medium.

resulting in a biomass concentration of 3493.3 ± 465.4 mg_{VS}/dm³ (Fig. 1). Chlorophyll a levels reached 3845.7 ± 696.2 µg/dm³ at $r = 349.61 \pm 63.29$ µg/dm³·d (Fig. 2). Nitrogen and phosphorus were found to have been efficiently removed from the growth medium, with their concentrations in the system being 0.39 ± 0.13 mg TN/dm³ and 0.02 ± 0.01 mg TP/dm³ (Figs. 3,4). Glucose supplementation in S2V2 did not significantly ($p = 0.05$) affect the *P. subcordiformis* growth rate (Table 4) – the biomass

concentration was 3671 ± 777.4 mg_{VS}/dm³ (Fig. 1). The r level was also similar to the value noted in S2V1 at 333.73 ± 70.67 mg_{VS}/dm³·d. Chlorophyll a level was 1848.3 ± 120.2 µg/dm³ (Fig. 2). Nitrogen take-up by the microalgae was close to $67.83 \pm 1.77\%$ (Fig. 7), which translates to the final N level of 6.89 ± 0.37 mg TN/dm³ (Fig. 3). N removal was at 4.32 ± 0.93 mg TN/g_{VS} (Fig. 5). The phosphorus concentration at the end of the experiment was 1.05 ± 0.06 mg TP/dm³ (Fig. 4), with the removal rate being

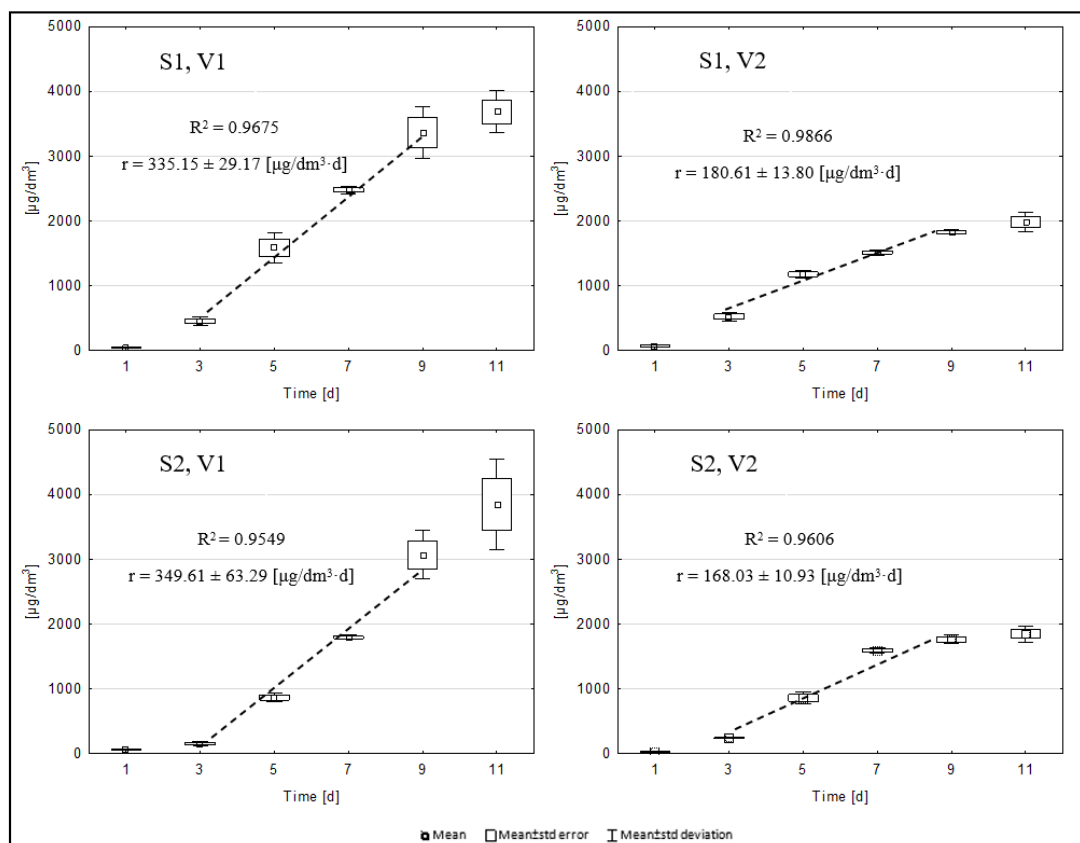


Fig. 2. Trends in chlorophyll a levels.

$79.97 \pm 1.26\%$ (Fig. 8) and phosphorus take-up per specific biomass growth being 1.25 ± 0.24 mg TP/g_{V_S} (Fig. 6). Phosphorus take-up was statistically comparable between the two variants (Table 4). Glucose concentration in the growth medium is presented in Fig. 9.

4. Discussion

Use of natural water as growth medium is a novel area of research that very few papers have dealt with so far [31]. Most publications focus on producing *P. subcordiformis* biomass on media prepared from distilled water and chemical reagents that provide optimal conditions for microalgal growth [24].

Water from Bay of Gdańsk was chosen in order to determine whether natural water is a viable medium for high-volume production of *P. subcordiformis* biomass. The final concentration was 3493.3 ± 465.4 mg_{V_S}/dm³ at a growth rate of 317.58 ± 42.31 mg_{V_S}/dm³·d. This biomass concentration is similar to those achieved by other researchers using synthetic and strictly controlled culture conditions on media prepared with distilled water and chemical reagents. Xie *et al.* [32] have reported 3680 mg_{V_S}/dm³ of the final *P. subcordiformis* biomass on the same type of medium, while Ji *et al.* [30] achieved a biomass production of 3200 mg_{V_S}/dm³ under similar conditions.

The study found that the waters from the coastal zone

of the Baltic Sea were too nutrient-deficient to support rapid growth of *P. subcordiformis* biomass. Therefore, the medium had to be supplemented with nitrogen and phosphorus from external sources. Ran *et al.* [29] reported similar findings after growing *P. subcordiformis* in a medium prepared with water from the southern part of Bohai Bay (China). This led to the conclusion that a satisfactory rate of algal growth can only be achieved with micronutrient supplementation. The final biomass levels in the study ranged from 1.85 to 2.0×10^6 cells/cm³ [29].

There are also literature reports confirming that *Platymonas sp.* microalgae can be grown in a blend of municipal sewage and industrial (textile) effluent [33]. The chlorophyll levels were found to be 2.8 µg/cm³ for *Platymonas suecica* and 7.3 µg/cm³ for *Platymonas chuii*. Another study also achieved high rates of nutrient take-up from aquaculture wastewater, with nitrogen and phosphorus removal in the ranges of 87.0–95.0% and 98.0–99.0%, respectively [34]. These nutrient take-up rates are similar to those achieved in our own research. Almost 100% of nutrients was utilized in the non-glucose-amended variants. In variants with external carbon source, the nutrient take-up was significantly lower. Possibility of recycling the culture medium composed in sea water for further microalgae cultivation was also investigated. Recirculation is often limited by the increase in salinity due to evaporation and contam-

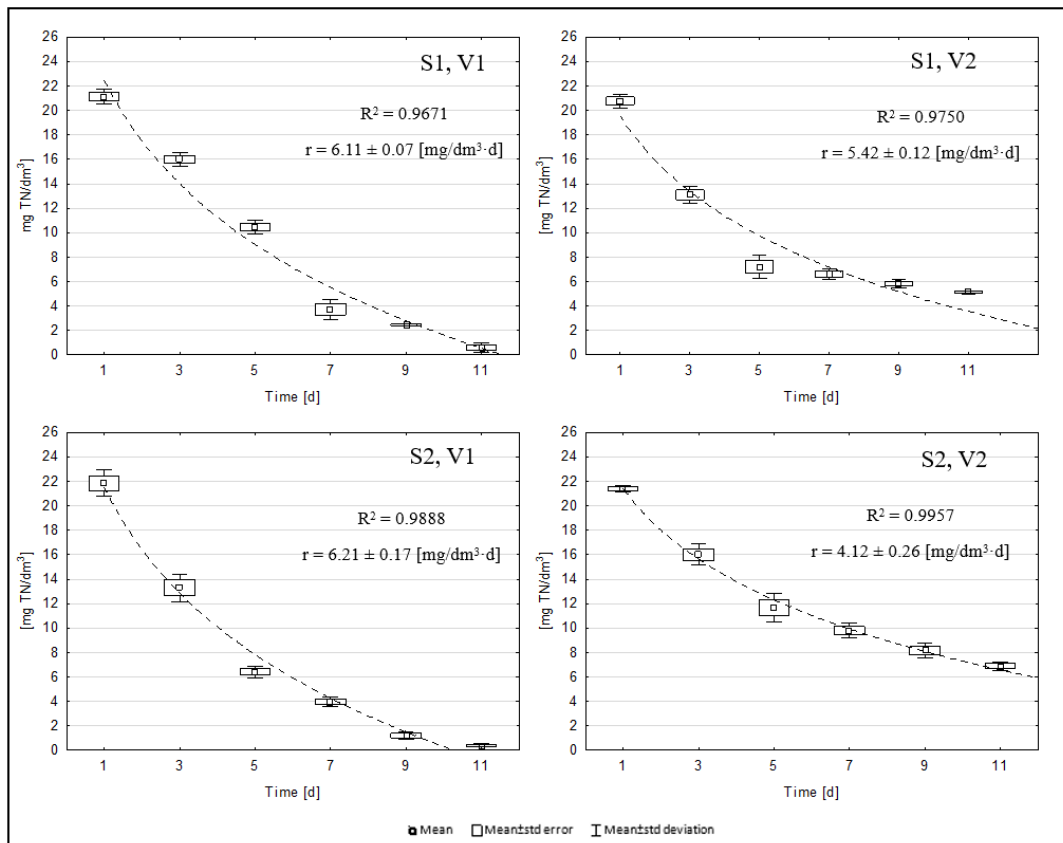


Fig. 3. Trends in TN concentration in the growth medium.

ination. It was proved that despite the increase in salinity from 5.5% to 12% (w/v), microalgae produced 48–160% more dry organic matter. The peak productivity was 37.5 ± 3.1 g VS m^2/day . The combination of mixotrophic growth ensuring high biomass yield under conditions of high salinity has been proven to be an effective sustainable cultivation strategy [35].

Most studies use closed culture systems due to the need to monitor and culture multiple process parameters that directly affect microalgal growth in hydrogen production systems [36]. Most literature reports focus on PBRs with active volumes of 250–5000 cm^3 [37]. A *P. subcordiformis* biomass grown by Guo *et al.* [38] in a column reactor reached levels that correspond to a phytoplankton concentration of 4.0×10^6 cells/ cm^3 , which translates to algal mass levels of 2500 mg_{TS}/dm^3 . Ji *et al.* [39] obtained a *P. subcordiformis* biomass concentration of 1800 mg_{TS}/dm^3 in a study conducted in an airlift tubular reactor with a working volume of 500 cm^3 . Given the strictly controlled nature of microalgal biomass production, this set-up enabled multiparametric monitoring of the culture [36,40]. One example of this design is the PBR used by Ji *et al.* [30] to cultivate *P. subcordiformis*. The authors obtained a microalgal biomass concentration of 3200 mg_{TS}/dm^3 .

In the present study, we also utilized a bioreactor capable of monitoring the process parameters important for microalgal cultivation, and grew a culture of *P. subcordiformis*. The final biomass concentration exceeded 3200 mg_{VS}/dm^3 – being significantly higher than the values reported by the above-cited authors. Xie *et al.* [32] used a similar type of PBR to produce similar levels of *P. subcordiformis* biomass at 3680 mg_{TS}/dm^3 . The culture was grown under mixotrophic conditions with glucose as an exogenous source of carbon. Initial glucose levels in the medium were 24 g/dm^3 [32]. In the present experiment, initial glucose level chosen was lower and reached 10 g/dm^3 . This decision was supported by the results of preliminary studies, in which attempts to test higher doses of glucose led to high turbidity of the culture medium. This blocked the light and thus directly arrested the growth of *P. subcordiformis*. Our own research showed that the final *P. subcordiformis* biomass levels fell within the relatively narrow range of 3200 to 3790 mg_{VS}/dm^3 across all experimental variants. However, the trends in biomass concentration in the PBR were different when chlorophyll a levels were used to measure this parameter. Variants with glucose dosing had nearly 50% lower concentrations of *P. subcordiformis* biomass measured by chlorophyll a levels compared to variants with no external carbon source.

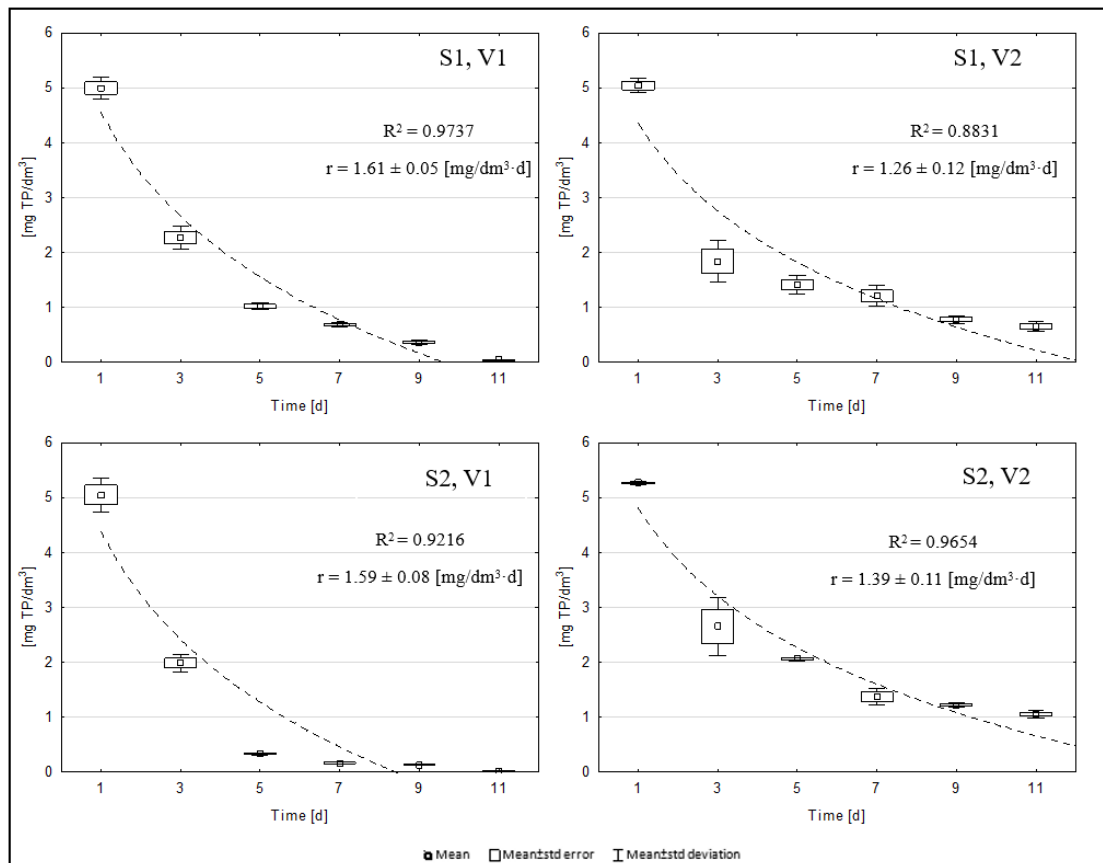


Fig. 4. Trends in TP concentration in the growth medium.

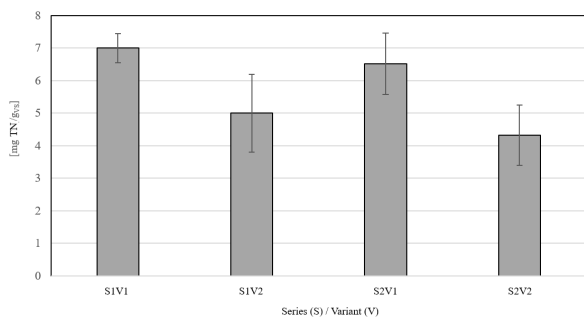


Fig. 5. TN take-up per specific gain of *P. subcordiformis* VS.

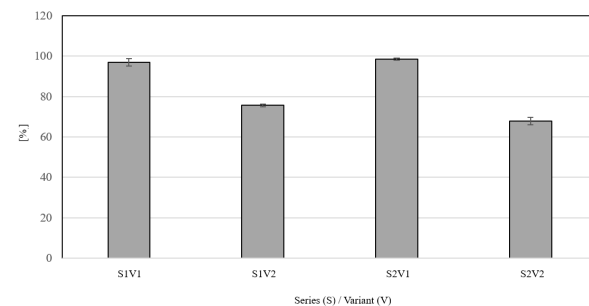


Fig. 7. Efficiency of TN removal by *P. subcordiformis*.

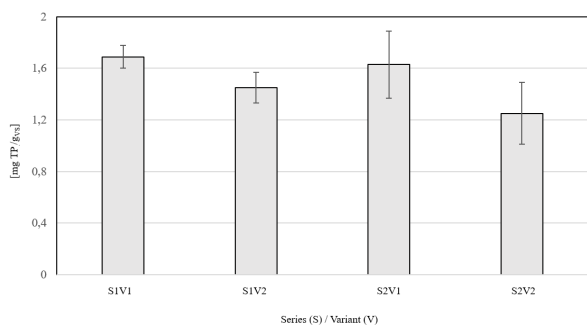


Fig. 6. TP take-up per specific gain of *P. subcordiformis* VS.

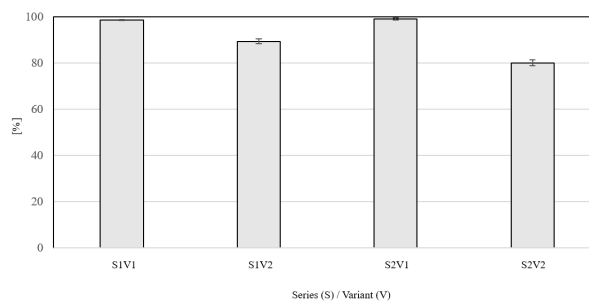


Fig. 8. Efficiency of TP removal by *P. subcordiformis*.

Table 4. Results of the Tukey HSD comparative analysis.

Experimental series/variant	Nutrient take-up per specific gain of <i>P. subcordiformis</i> VS							
	TN				TP			
	S1V1	S1V2	S2V1	S2V2	S1V1	S1V2	S2V1	S2V2
S1-V1		0.070680	0.993059	<i>0.011031</i>		0.653611	0.984588	0.236786
S1-V2	0.070680		0.168430	0.869060	0.653611		0.943876	0.951561
S2-V1	0.993059	0.168430		<i>0.027432</i>	0.984588	0.943876		0.538081
S2-V2	<i>0.011031</i>	0.869060	<i>0.027432</i>		0.236786	0.951561	0.538081	

Experimental series/variant	Efficiency of nutrient removal by <i>P. subcordiformis</i>							
	TN				TP			
	S1V1	S1V2	S1V1	S1V2	S1V1	S1V2	S1V1	S1V2
S1-V1		<i>0.000159</i>	0.991833	<i>0.000159</i>		<i>0.000159</i>	0.991833	<i>0.000159</i>
S1-V2	<i>0.000159</i>		<i>0.000159</i>	<i>0.027120</i>	<i>0.000159</i>		<i>0.000159</i>	<i>0.027120</i>
S2-V1	0.991833	<i>0.000159</i>		<i>0.000159</i>	0.991833	<i>0.000159</i>		<i>0.000159</i>
S2-V2	<i>0.000159</i>	<i>0.027120</i>	<i>0.000159</i>		<i>0.000159</i>	<i>0.027120</i>	<i>0.000159</i>	

Experimental series/variant	VS and chlorophyll in growth medium at the end of the growth period							
	VS				chlorophyll a			
	S1V1	S1V2	S1V1	S1V2	S1V1	S1V2	S1V1	S1V2
S1-V1		0.998996	0.975066	0.843580		<i>0.000494</i>	0.989136	<i>0.000307</i>
S1-V2	0.998996		0.998996	0.961028	<i>0.000494</i>		<i>0.000290</i>	0.994234
S2-V1	0.975066	0.998996		0.997306	0.989136	<i>0.000290</i>		<i>0.000220</i>
S2-V2	0.843580	0.961028	0.997306		<i>0.000307</i>	0.994234	<i>0.000220</i>	

* – values in italics are significant at $p \leq 0.05$.

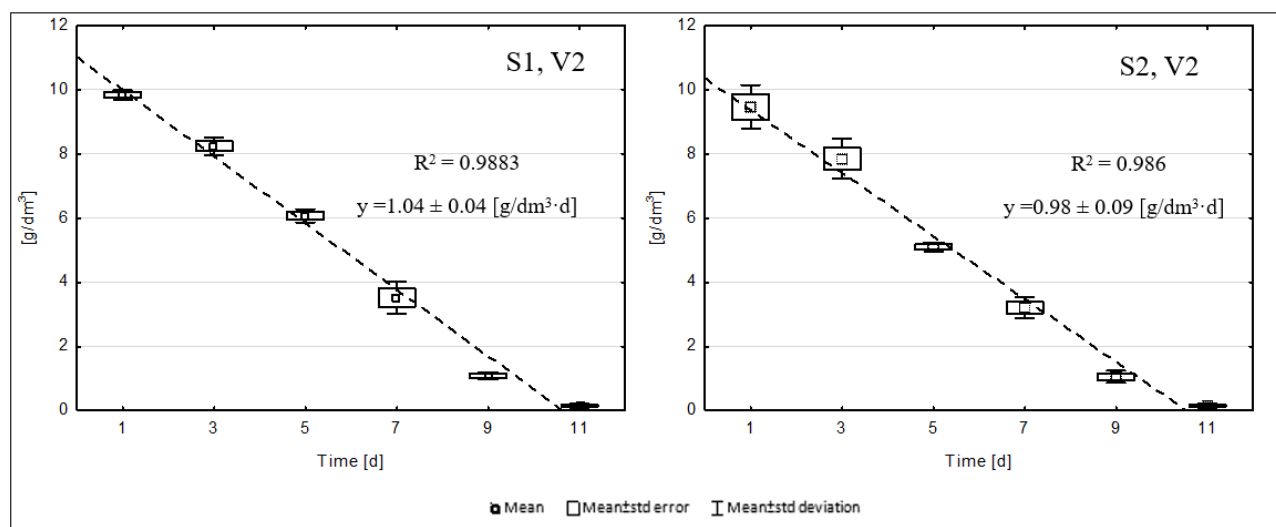


Fig. 9. Changes in glucose levels in the growth medium.

Xie *et al.* [32] also found decreased chlorophyll content in *P. subcordiformis* cultures stimulated by added glucose. The chlorophyll content in the photoautotrophic culture (without external carbon source) was, on average, twice as high than in the mixotrophic culture with added glucose [32]. A similar trend was observed by Faraloni *et al.* [41] in a *Chlamydomonas reinhardtii* culture grown in a medium made from wastewater rich in organic acids and carbohydrates. Chlorophyll content was 25 mg/dm^3 , be-

ing significantly lower than in the control microalgae culture grown in a mineral medium (50 mg/dm^3 chlorophyll a). The culture was grown mixotrophically at 28°C and exposed to $70 \mu\text{mol E/m}^2 \cdot \text{s}$ light. In the control, the chlorophyll concentration grew throughout the experiment (70 h) and stabilized only at the last stage of growth. In contrast, the stabilization occurred just after 20 h in the culture supplemented with industrial wastewater, with the biomass remaining stable afterwards [41].

Fluctuations in the level of photosynthetic pigments can occur in response to changes in microbial metabolism in photoautotrophic and mixotrophic conditions. In the autotrophic culture, microalgal growth is fuelled directly by photosynthesis (which in turn is driven by light energy). In mixotrophic environments, an additional source of energy is provided in the form of an external carbon substrate (easily degradable organic compounds). However, this additional energy source inhibits photosynthesis, which stimulates the formation of pigments in microalgae such as *P. subcordiformis* [42].

4. Conclusions

Growing *P. subcordiformis* in water sourced from the Bay of Gdańsk led to high biomass growth rates, comparable with those observed when using growth media based on deionized water and chemical reagents. It was found that water sourced from the coastal zone of the Baltic Sea was too nutrient-deficient to support rapid growth of *P. subcordiformis* biomass, therefore, the medium had to be supplemented with nitrogen and phosphorus from external sources. Amendment of the growth medium with glucose significantly limited chlorophyll production in microalgal cells, thus resulting in lower chlorophyll levels than in the variants with no external carbon source. The glucose-amended variants had significantly higher nitrogen and phosphorus levels in the medium at the end of the growth period. The use of mixotrophic culture allowed to obtain significantly higher efficiency of *P. subcordiformis* culture grow characterized by the final concentration of dry organic matter. The use of natural sea waters has been proven to be justified from a technological and economic point of view.

Author contributions

MDu and MDę designed the research study. MDu and AN performed the research. MZ provided help and advice. MDu, MDę and MZ analyzed the data. MDę and JK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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