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Potato starch production side stream is a suitable medium for microalgae cultivation

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ABSTRACT

Agro-industrial side streams can provide alternative substrates for microalgae cultivation and sustainable production of valuable bioproducts through recycling and re-use of limited resources. This work explored the potential for using a side stream from potato starch production (evaporation-concentrated potato cell fluid, potato juice) as an alternative medium for scaled-up cultivation of microalgae. A high-throughput screening approach was employed to identify strains of green algae that can grow in the side stream. The selected candidate strains were tested further in optimization experiments to determine the suitable side stream concentration and optimal light intensity. Finally, two strains with the highest specific growth rates in the side stream, *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA-CHL 103) were cultivated in 3 L flat-panel photobioreactors. The biomass was harvested and assessed for the content of carotenoids, fatty acids and amino acids. The 1 % potato juice medium supported growth rates and biomass yield comparable to the commercial algal media, with similar biochemical profiles of the harvested biomass. These results demonstrate that microalgae biotechnology can be employed to valorise unexploited side streams from potato starch production, adding a circular value to this agro-industrial process.

1. Introduction

Microalgae are a highly diverse group of primarily photosynthetic protists that represent a promising biotechnological resource [1]. Due to their relatively simple growth requirements, microalgae are suitable for large-scale production of biomass and commercially valuable bioproducts [2]. However, the supply of nutrients remains a significant constraint for large-scale commercial cultivation, increasing the production costs and limiting the integration of microalgal biotechnology into bioeconomy pathways [3]. This issue has prompted the search for alternatives to custom-made algal growth media, with various types of agro-industrial residues and side streams being considered as suitable sources of nutrients for microalgae cultivation [4,5].

Potato agriculture covers ~1,7 million hectares in the European Union, producing ~48,3 million tonnes of potatoes per year (data from 2023; [6]). The harvested biomass is processed into various products, the most relevant being frozen potatoes, dried potatoes, prepared or preserved potatoes and potato starch. As such, the potato industry is a major source of various agro-industrial residues, which can provide valuable resources for the circular bioeconomy [7]. Due to its abundance and availability, the liquid side stream from the starch extraction process has been used for a variety of purposes. For example, concentrated potato residue has been utilized as a fertilizer [8], while microbial processing has been applied for the production of biogas [9], biochemicals like ethanol [10] and lactic acid [11], as well as single cell *Bacillus* protein (SCP) for animal feed [12,13].

Earlier studies have demonstrated that microalgae can be grown at large-scale in plant-based, organic-rich side streams and agro-industrial residues. Media based on fruit and horticultural biocompost were proved to be suitable for growing a range of microalgae, e.g. the commercially interesting genera *Chlorella, Chlamydomonas* and *Lagerheimia*, which reached higher lipid and protein contents when grown in side streams [14–16]. Microalgae capable of mixotrophic growth, such as *Chlorella*, that utilise both inorganic micro-and macro-nutrients and organic compounds from the medium are particularly interesting in this context [17]. Specific challenges were also identified when growing

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algae in organic-rich residues, mainly related to contamination by associated microorganisms as well as inconsistent quality and varying nutrient content of supplied residues [18]. As a rich source of nutrients and various organic carbon compounds, wastewater from potato industry has also been successfully tested in algae cultivation for production of pigments [19]. Clearly, there is a need for further research in this direction, focusing on wider screening of algae strains, optimization of the cultivation process and detailed assessment of produced biomass.

This work explores the potential for using evaporation-concentrated potato cell fluid (here referred to as "potato juice (PJ)") as an alternative medium for microalgae cultivation, with subsequent biochemical characterisation of algal biomass focusing on commercially valuable bioproducts (carotenoid pigments, fatty acids and amino-acids). The work follows these main lines: (I) Chemical characterisation of PJ and its adjustments for microalgae cultivation; (II) High-throughput screening of microalgae strains from the Norwegian Culture Collection of Algae (NORCCA) suitable for cultivation in PJ; (III) Optimization of PJ formulation and light conditions for scaled-up cultivation; (IV) Scaled-up cultivation of best-growing strains in flat panel bioreactors; (V) Chemical characterisation and assessment of the obtained biomass. Demonstrating the feasibility of PJ as an alternative cultivation medium can open new opportunities for circular bioeconomy based on side-streams from the potato starch industry and generate sustainable microalgal biomass rich in commercially interesting bioproducts.

2. Material and methods

2.1. Potato juice: origin and physico-chemical properties

Evaporation-concentrated potato cell fluid was obtained from Finnamyl Oy, Kokemäki (Finland). For preparation of the starch and protein fraction and the cell fluid, the starch potatoes were washed and rasped carefully to get all the fruit juice out of potato cells. All solids were removed, after which the potato starch was separated from the fruit juice. The temperature and pH were adjusted appropriately in the remaining fruit juice to coagulate the protein. The coagulated protein was washed, and the remaining potato cell fluid was evaporation concentrated. The cell fluid (potato juice (PJ)) was divided in 250 mL plastic bottles, frozen and stored at −20 °C before its use in the growth experiments.

Initial chemical profile of the potato juice was provided by the supplier. Chemical analysis of selected elements (K, Ca, Mg, P, Fe, S and total N) in 1 % potato juice (PJ) medium batch used in the scale-up experiment was done using ICP-MS and Anion-Kation analysis. Measurements of nitrate and phosphate concentrations were conducted on the 1 % PJ medium using Hach spectrophotometer kits TNT835 and TNT843, respectively (Hach, United States).

2.2. High-throughput screening of candidate strains

In total, 16 unialgal (non-axenic) strains of freshwater green algae (Chlorophyta) from the Norwegian Culture Collection of Algae (NORCCA) were tested for growth in the PJ-based media (Table 1). The selected strains included genera and species that commonly grow in media based on agro-industrial residues and are known producers of commercially interesting bioproducts (e.g. *Chlorella*, *Haematococcus*, *Scenedesmus* etc.).

For the screening experiments, the potato juice was defrosted overnight at 4 °C. After defrosting, the PJ was autoclaved (121 °C/15 min) and refrigerated for three days (4 °C) until the larger particles from the juice were sedimented. Then, the PJ was decanted, separating the liquid component from the sedimented particles. The decanted fraction was stored at 4 °C and used in subsequent high-throughput screening and growth optimization experiments. In this work, the PJ was utilized as a concentrated stock solution, which was diluted with dH_2O to prepare "PJ media" of different concentrations. While the PJ was sterilized during the autoclave treatment, all subsequent handling of PJ during experimental work was conducted in non-sterile conditions.

Table 1
List of 16 green algal strains from NORCCA used in the high-throughput screening phase.

Species	NORCCA code	Isolation habitat
Chlorococcum sp.	NIVA-CHL 131	Terrestrial, biofilm-forming
Chlorococcum sp.	NIVA-CHL 103	Freshwater, river
Chlamydomonas noctigama	NIVA-CHL 168	Terrestrial, soil
Chlamydomonas sp.	NIVA-CHL 143/1	Freshwater, "red snow"
Haematococcus pluvialis	K-0084	Freshwater, rock pool
Chlorella sp.	NIVA-CHL 15	Terrestrial, house dust
Chlorella vulgaris	NIVA-CHL 19	Terrestrial, house dust
Scenedesmus sp.	NIVA-CHL 133	Freshwater, swimming pool biofilm
Desmodesmus subspicatus	NIVA-CHL 55	Freshwater, aquarium
Desmodesmus armatus	K-1612	Freshwater, lake
Tetradesmus cf. *dimorphus*	NIVA-CHL 182	Freshwater, lake
Tetradesmus obliquus	NIVA-CHL 6	Freshwater, lake
Monoraphidium griffithii	NIVA-CHL 8	Freshwater, lake
Monoraphidium convolutum	NIVA-CHL 30	Freshwater, lake
Choricystis cf. *coccoides*	NIVA-CHL 88	Freshwater, lake
Raphidocelis subcapitata	NIVA-CHL 1	Freshwater, river

Algae were tested for growth in four PJ-based media: 0.1 % and 1 % PJ in dH2O (here referred to as "0.1% PJ medium" and "1% PJ medium", respectively), 1 % PJ in stock Z8 medium ("1 % PJ-Z8"), and pure Z8 medium [20]. Preliminary observations have revealed that 0.1 % PJ medium has similar transparency to the stock Z8 medium and chemical analysis showed that it contains sufficient concentrations of macronutrients for algal growth (Table 2). On the other hand, 1 % PJ medium is richer in nutrients but is more turbid and may limit the light available for algal growth. Finally, the aim of using 1 % PJ-Z8 medium was to observe whether compounds present in Z8 medium but not in the PJ are limiting algal growth in 1 % PJ medium.

The screening experiment was conducted in 96-well plates. Experimental conditions were identical to the growth conditions of the stock cultures in the NORCCA collection, with 15 °C temperature, 10 μmol photons m^{-2} s^{-1} light intensity, and 14:10 h of light:dark period in order to minimize the need for acclimation. Stock cultures of candidate strains were acclimated to growth in well plates by growing in stock Z8 medium in 24-well plates (2 mL volume) for one week. Then, 10 μL of dense acclimated culture was inoculated in 290 μL of test media (0.1 % PJ, 1 % PJ, 1 % PJ-Z8 and Z8) in three replicates. The well plates were sealed with transparent sealing film (ThermoFisher, United states) to prevent evaporation while allowing for gas exchange. The fluorescence values from each well were measured daily using Cytofluor 2300 plate reader (Millipore, United States), programmed to measure the fluorescence of green algae using 485 nm excitation wavelength and 685 nm emission wavelength.

Table 2
Chemical analysis of potato juice after starch extraction.

Compound	Concentration (g kg^{-1})
Total Nitrogen (N)	38 (± 7.7)
Soluble Nitrogen (N)	26
Ammonium (NH4-N)	1.9
Total Phosphorus (P)	9.5 (± 1.4)
Potassium (K)	120 (± 25)
Magnesium (Mg)	8.0 (± 1.6)
Calcium (Ca)	0.83 (± 0.21)
Copper (Cu)	0.0067 (± 0.002)
Manganese (Mn)	0.038 (± 0.01)
Zinc (Zn)	0.079 (± 0.024)
Sodium (Na)	6.6 (± 2.7)
Boron (B)	0.024 (± 0.0073)

2.3. Growth optimization experiment

The optimization experiment aimed to determine the optimal dilution of potato juice and optimal light intensity, focusing on four algal strains selected in the screening experiment: *Chlorella* sp. (NIVA-CHL 15), *Chlorococcum* sp. (NIVA-CHL 103), *Tetradesmus obliquus* (NIVA-CHL 6) and *Raphidocellis subcapitata* (NIVA-CHL 1). As the turbidity increases along with nutrients at higher potato juice concentrations, growth response was evaluated across a gradient of potato juice concentrations and irradiance levels. Light intensity was varied to distinguish nutrient limitation from light limitation, and to determine if increased light intensity can compensate for the increasing turbidity of the potato juice. The tested solutions of potato juice in dH2O were 0.1 %, 1 %, 2 % and 3 %, and the tested light intensities tested were 10, 25, 50, 100, 150, 200, 250 and 300 μmol photons m^{-2} s^{-1}.

The optimization experiment was conducted in 96-well plates, using a high-throughput "nannocosm" approach [21]. Here, a custom-made light panel (Maplebear electronics, USA) with 96 customisable LED lights provided each well with a white-light source of specific light intensity. The stock cultures were acclimated to the culture collection conditions, at 15 °C temperature under 10 μmol photons m^{-2} s^{-1} light intensity and 14:10 h of light:dark cycle for two weeks in 24 well-plates. At the start of the experiment, 1 μL of dense stock culture was inoculated into 299 μL of each PJ medium formulation and Z8 medium. The inoculated plates were sealed with BreatheEasy transparent seal (Diversified Biotech, United States), covered with LED light panels, and kept at 15 °C with 14:10 h light:dark cycle. Measurements were taken daily on Synergy Mx plate reader (BioTek, United States) using the same excitation and emission wavelengths as in the screening experiment.

2.4. Scaled-up cultivation of selected strains

The two strains that demonstrated the best growth in potato juice during the screening and optimization experiments, *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA CHL 103) were scaled-up in flat panel bioreactors. The aim of the experiment was to compare growth and the chemical composition of the biomass in 1 % PJ medium with the stock medium Z8. The potato juice was first autoclaved (121 °C/15 min) and then centrifuged at 2500*g* for 10 min to sediment larger particles. The supernatant was then decanted and mixed with dH_2O to prepare a 1 % PJ solution which was vacuum filtered through GF/C (pore size 1,2 μm) and GF/F (pore size 0,7 μm) filters. The aim of the filtration steps was to remove the particulate organic matter from the medium and thus minimize the growth of bacteria and heterotrophic protists during scale-up. Also, it ensured more precise measurements of dry algal biomass and was intended to improve the transparency of the medium. Stock Z8 medium was prepared by adding standard stock solutions to dH_2O and filtering the medium through GF/C and GF/F filters. The filtered media were stored in 20 L plastic containers and kept at 4 °C. The media were then acclimated to 20 °C before the inoculation of algal stock cultures.

The scale-up experiment was conducted in a flat panel bioreactor system (Supplementary Fig. 2). Each flat panel bioreactor was made from a heat-sealed plastic bag, size adjusted to fit 3 L of culture. The panels were 10-15 cm thick to ensure a short light path. Light was provided by four vertical LED light panels. The dense inoculum was pre-cultivated in 3 L volume of Z8 medium at 20 °C, 16:8 h light:dark cycle and ~200 μmol photons m^{-2} s^{-1} light intensity. At the beginning of the experiment, 1 L of dense inoculum (in a late exponential growth phase) was mixed with 13 L of each medium (1 % PJ and Z8 stock medium), mixed thoroughly, sampled for initial biomass measurement and distributed into four 3 L replicates. Each flat panel replicate was equipped with an air-bubbling system, and pH was kept stable at 7.5 through CO_2 injection. Daily measurements were collected for dry biomass weight and optical density measurements, along with routine inspection of cultures under light microscope, tracking the experiment progression, microalgal growth and potential biological contaminants. For cell dry weight (CDW) measurements, a fixed culture volume (10-50 mL depending on growth stage) was filtered on a pre-combusted and pre-weighted Whatman glass microfiber GF/C filters and dried overnight in oven at 50 °C before weighing again to determine CDW. Optical density (OD) was measured using Hach DR3900 Laboratory Spectrophotometer (Hach, United States) at absorbance of 750 nm. Once the cultures reached the stationary phase, a 600 mL volume from each bioreactor was collected and biomass was harvested by centrifugation. The biomass was then stored at −20 °C, and freeze dried in Christ Alpha 2–4 LD Freeze Drier (Martin Christ, Germany) for the following chemical analysis.

2.5. Chemical analysis of obtained biomass

About 500 mg of dried microalgal biomass from each replicate was used for the biochemical profile assessments. The algal FAME profile was analysed using the method described by O'Fallon et al. (2007), in the Trace GC Ultra multi-channel gas chromatograph with auto injector (Thermo Scientific, United States) at LabTek (Norwegian Institute of Bioeconomy Research (NIBIO), Norway). The amino acid profile (excluding tryptofane) was determined using 30+ Amino Acid Analyser (Biochrom Ltd., Cambridge, England), also at LabTek. Carotenoid analysis was performed on HPLC at Nutrition Analytical Service (Institute of Aquaculture, University of Stirling, UK).

2.6. Analysis of growth parameters

Daily fluorescence measurements expressed as Relative Florescence Units (RFU) were imported using an automated script in R Studio (*v. 4.3.1*). Blank reads of each medium were measured using same settings and subtracted from the fluorescence data. Maximum growth rate (μmax) in each well was calculated using "all_splines" method in the R package *growthrates* [22](Supplementary Fig. 3). *t*-tests were performed using the *broom* package in RStudio to evaluate the significance of observed differences in growth rates between 1 % PJ medium and Z8 for both species, separately for each estimation method (OD and CDW).

2.7. Statistical analysis of biochemical data

Media effects on bioproduct concentrations were analysed within each strain. For amino acids (g kg^{-1}) and carotenoids (mg kg^{-1}), concentrations were log transformed and modelled as: log(conc) ~ analyte + media + analyte:media + (1|culture), with a random intercept for culture to account for multiple analytes measured on the same culture. Media effects (1 % PJ vs Z8) were obtained from estimated marginal means contrasts and back transformed to PJ: Z8 geometric-mean ratios with 95 % confidence intervals. *P*-values were adjusted within strain across analytes using the Benjamini and Hochberg procedure. For fatty acids (percent of total), aggregate totals were removed, and profiles were treated as compositions: for each strain, data were analysed on the centered log ratio scale using clr ~ analyte + media + analyte:media. PJ vs Z8 contrasts were estimated only for analyte–strain combinations with nonzero proportions in both media and are reported as exp.(ΔCLR), the multiplicative change in relative abundance; *p*-values were adjusted within strain using the same procedure. Diagnostic checks (residuals and Q–Q plots) indicated adequate fit. Descriptive tables report observed means ± SD per strain × media for context and statistical inference follows the models (Supplementary Tables 2–7). Analyses were performed in R with *lme4* and *emmeans*. Data handling and plotting used *dplyr*, *tidyr*, *forcats*, *ggplot2*, and *cowplot*.

3. Results and discussion

3.1. Physical and chemical characteristics of the potato juice

Potato juice (PJ) appeared as a viscous, dark liquid, with high

concentration of suspended solids. High turbidity and low light penetration observed in PJ are common features of organic-rich side streams, and are often related to high concentrations of various humic and tannic acids [23]. The elimination of suspended particles through sedimentation and decanting, followed by filtration through series of filters with decreasing mesh size, as well as subsequent autoclave treatment, did not alter the transparency of PJ. Ultimately, dilution of PJ in water was necessary to obtain a medium with light penetration suitable for microalgal growth (Supplementary Fig. 1A).

Concentrations of N and P in PJ were sufficiently high to formulate a PJ-based algal growth medium through dilution. Indeed, following a 1:100 dilution in dH_2O, the 1 % PJ medium had a similar nutrient profile as the stock medium Z8 [20, Supplementary Table 1] and greatly improved transparency that enabled for microalgal growth (Table 2, Table 3). Specifically, the concentration of nitrate in 1 % PJ medium (23.2 mg L^{-1}) was lower than in Z8 (83.9 mg L^{-1}), and the phosphate concentration in 1 % PJ medium was one order of magnitude higher (~50 mg L^{-1} compared to 5,5 mg L^{-1} in Z8). A range of macro- (e.g. potassium (K), magnesium (Mg), calcium (Ca), sulphur (S)) and micro-nutrients (iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), sodium (Na) and boron (B)) essential for algal growth [24,25] were also sufficiently abundant in 1 % PJ medium (Tables 2 and 3).

Alongside macro- and micro-nutrients, PJ is typically abundant in a variety of organic molecules that can favor the growth of mixotrophic microalgae. For example, amino acids can constitute up to 10 % of potato juice dry matter, and are known to improve mixotrophic growth rates in microalgae [26–28]. Sugars such as sucrose, glucose and fructose, which can reach total concentrations of up to 10 g L^{-1} in PJ, are key organic carbon sources for mixotrophic or heterotrophic microalgae [29]. Other, more complex organic molecules abundant in PJ such as proteins (up to 30 % dry matter, [26]) and starch are not immediately bioavailable for microalgae, and various pre-treatments of PJ are needed before their use in microalgae cultivation [30].

3.2. High throughput screening experiment

This study has validated the use of well plate-based high-throughput screening approach for identifying microalgal strains capable of growing in various agro-industrial side streams. Most importantly, well-plate based methods allow for parallel testing of a large number of strains, side stream or medium formulations or growth conditions as well as for further optimization of temperature, light, side stream dilutions and nutrient concentrations [21,24].

In the initial screening experiment, all the microalgal strains exhibited growth in at least one of the three tested PJ formulations (Fig. 1, Fig. 2). The formulation that was favourable for most of the strains was 0.1 % PJ medium, in which all the tested strains could grow, although with varying maximum growth rates. The highest growth rates in the 0.1 % PJ medium were observed in *Raphidocelis subcapitata* (NIVA-CHL 1; μmax = 0.25) and in *Tetradesmus obliquus* (NIVA-CHL 6; μmax = 0.21), which were selected for the subsequent optimization experiment. In nearly all strains, maximum observed growth rates were higher in 0.1 % PJ medium than in the stock Z8 medium. While 1 % PJ medium was found to be suitable for only a few strains, it supported the highest growth rates observed in the whole screening experiment, with *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA-CHL 103), reaching maximum growth rates of 0.37 and 0.45, respectively, the highest growth rates observed in the entire screening experiment. Due to their exceptionally high growth rates in more nutrient-rich 1 % PJ medium (Fig. 2), and high fluorescence values indicating higher biomass yield (Fig. 1), these two strains were also selected for optimization experiment. For most of the strains, additional nutrients provided in the 1 % PJ-Z8 medium did not stimulate growth, suggesting that the increased turbidity of this medium combined with relatively low light intensity could have been the limiting factor for algal growth. Clearly, PJ-based media were demonstrated to be highly suitable for microalgae cultivation, confirming the findings of earlier studies [19,31,32].

While green algae from *Chlorella, Chlorococcum, Scenedesmus* and *Tetradesmus* genera have previously been demonstrated to grow in various agro-industrial side streams [33–35] including PJ-based media [35], our study shows that capacity for growth in more concentrated side streams is strain-specific, as indicated earlier by Yuan et al. [19]. For example, different strains of *Chlorella* and *Chlorococcum* showed both the highest and the lowest growth rates in 1 % PJ medium (Fig. 2) during our screening. This highlights the importance and value of culture collections and inclusion of broader microalgal diversity in the screening process, to account for strain-specific phenotypic and physiological variation.

The capacity to grow in PJ-based media is also likely related to pre-treatment procedures, which can render the potato juice more suitable for microalgae cultivation. For example, *Haematococcus pluvialis* strain showed a weak growth in PJ-based media formulated by dilution in this study, while other *H. pluvialis* strain was growing successfully in anaerobically digested potato juice in study by Pan et al. [31]. Another approach by Mohamadnia et al. [32] utilized anaerobic acidification of potato juice as pre-treatment before microalgae cultivation, with good results. Clearly, pre-treatment procedures need to be tailored to strains of interest and their bioproducts, ensuring both the efficient nutrient recovery from potato juice and production of microalgal biomass with desired bioproduct profiles.

Table 3
Macronutrient concentrations in the potato juice formulations measured using quick kits (Hach, United States), Anion-Kation analysis and IPC-MS, compared to known concentrations in the stock medium Z8 (Kotai, 1972).

	1 % PJ	Z8
Quick kit		
Total Nitrogen (mg L^{-1})	159	83.9
Nitrate (mg L^{-1})	23.2	83.9
Ammonium (mg L^{-1})	0.029	0
Phosphate (mg L^{-1})	55	5.5
TOT-N		
TIN (mg L^{-1})	5,4	83.9
ICP-MS		
Phosphate (mg L^{-1})	64.7	5.5
Sulphur (mg L^{-1})	73	3.25
Iron (μg L^{-1})	1030	580
Anion-Kation analysis		
Potassium (mg L^{-1})	1080	14
Calcium (mg L^{-1})	11.7	10
Magnesium (mg L^{-1})	64.1	2.5

3.3. Optimisation experiment

The optimization experiment provided better insight into effects of different PJ formulations and light conditions on growth of the strains selected in the initial screening (Fig. 3, Fig. 4). *Chlorella* sp. and *Chlorococcum* sp. grew best in the 1 % PJ medium, reaching slightly higher growth rates (0.45 and 0.5, respectively) than in the initial screening. Same as in the screening experiment, maximum fluorescence values (biomass) reached by *Chlorella* and *Chlorococcum* were higher in 1 % than in the 0.1 % PJ medium. Therefore, based on high growth rates and biomass accumulation in more nutrient-rich 1 % PJ medium, these two strains were selected for the scale-up experiment. Interestingly, increasing PJ concentrations to 2 % and 3 % did not improve the growth, possibly due to reduced light penetration in these media, an issue that could not be compensated by increasing light intensities or possible growth inhibition by more concentrated micro- and macro-nutrients [36]. Another possibility is that PJ contains certain compounds (e.g.

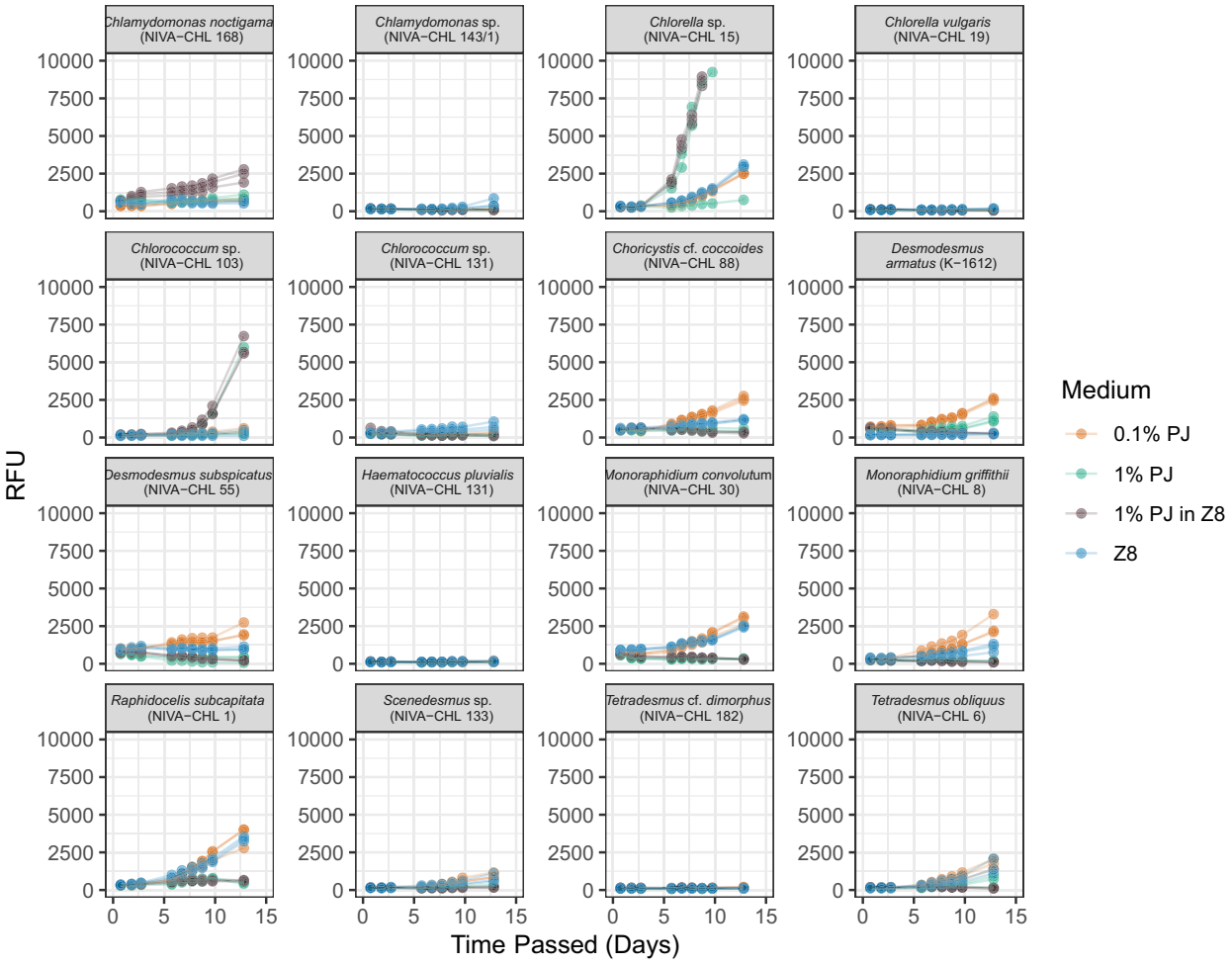

Fig. 1. Growth curves of Green algae strains grown in PJ-based media and control medium Z8 during the high-throughput screening experiment.

alkaloids and glycoalkaloids) that were inhibiting algal growth in more concentrated 2 % and 3 % PJ formulations [37,38].

Along with the highest growth rates in 1 % PJ medium, the two selected strains demonstrated comparable light preferences (Fig. 5). Both strains exhibited highest growth rates under the light intensity of around 100–200 µmol photons m^{-2} s^{-1}, while *Chlorella* sp. was seemingly more tolerant to light intensities higher than 200 µmol photons m^{-2} s^{-1}. It is important to note that the stated light intensities are not accounting for the lower light penetration in the 1 % PJ medium, and that the actual light intensity experienced by the cells was likely somewhat lower. In any case, observed light optima were generally in line with the available data for both species [39,40]. The two remaining strains, *Raphidocellis subcapitata* and *Tetradesmus obliquus* exhibited weak growth in 1 % PJ medium and inconsistent growth in 0.1 % PJ medium that did not warrant their use in the subsequent scale-up experiments.

3.4. Scale-up experiments

Scale-up experiments demonstrated that both *Chlorella* sp. and *Chlorococcum* sp. can be cultivated successfully in 1 % PJ medium, reaching growth rates and biomass yields comparable to the stock Z8 medium (Fig. 6, Supplementary Fig. 1B). The maximum biomass of *Chlorella* sp. at harvest was 1.3 g L^{-1} in 1 % PJ medium compared to 1.5 g L^{-1} in Z8 medium (Fig. 6B). However, CDW-based growth rates were significantly higher in Z8 medium, reaching the maximum of 0.68 compared to 0.43 in 1 % PJ medium. Differences in growth rates were even more pronounced when considering optical density data. Here, *Chlorella* exhibited maximum growth rate of 0.52 in Z8, compared to 0.19. in 1 % PJ medium. For both methods, differences in growth rates were statistically significant, indicating that *Chlorella* indeed grew faster in Z8, but has reached similar biomass concentrations by the end of the experiment. In *Chlorococcum* sp., maximum biomass yield at the time of harvest was 1.56 g L^{-1} compared to 1.29 in Z8 medium, with maximum observed growth rates of 0.66 observed both in 1 % PJ medium and in Z8 medium, based on OD measurements. Growth rates based on CDW showed slightly faster growth in Z8 (0.85 compared to 0.8 in 1 % PJ), although with a weak statistical significance. As in *Chlorella*, mean CDW-based growth rate was slightly higher in Z8, but eventually cells were harvested at similar biomass concentrations. The inconsistency in growth data and thus growth rates obtained from CDW and OD likely stems from high initial bacteria load in 1 % PJ medium. Bacteria were detected by OD signal (absorbance), thus dampening the algae concentration signal, but were then partially washed through the filters during CDW measurements.

The growth rates and biomass yield observed here were comparable to other studies in which *Chlorella* sp. and *Chlorococcum* sp. were cultivated in agro-industrial side streams or with addition of organic carbon source [17,41,42]. Commonly, both species exhibit highest growth rates when grown heterotrophically, and mixotrophic growth is commonly faster than autotrophic growth [17,41]. Due to abundance of organic carbon in potato juice [19,35], it is safe to assume that *Chlorella* and *Chlorococcum* were growing mixotrophically in 1 % PJ treatment, and autotrophically in Z8 which lacked organic carbon source. In this

Fig. 2. Heat map showing the maximum growth rate (μmax) detected for each strain grown in PJ-based media and control medium Z8, sorted by the highest measured growth rate.

context, slower growth rates in 1 % PJ medium under mixotrophic growth suggest that further optimization of scaled up cultivation in PJ-based media is needed to improve biomass production rates. Efforts should focus on identifying possible inhibitory effects of various bio-compounds or trace elements found in PJ, and regulating bacterial load to minimize competition for organic carbon. Ultimately, heterotrophic cultivation in bacteria-free PJ should be explored for *Chlorella*, as it may provide faster growth rates and eliminate the issue of light penetration in more concentrated PJ formulations [43,44].

Despite comparable biomass yields and growth rates, notable qualitative differences in PJ medium and Z8 medium cultures were observed by light microscopy during the experiments. In the initial days of scaled-up experiments, 1 % PJ medium cultures of both *Chlorella* sp. and *Chlorococcum* sp. exhibited an increased production of bacteria and heterotrophic flagellates. However, this contamination waned after day 4 in *Chlorella* sp. batches, which were almost free from flagellate contamination by the sampling day (estimated >95 % of biomass was *Chlorella* cells as observed under microscope). In *Chlorococcum* sp. cultures, bacterial biomass was higher in 1 % PJ medium batches compared to Z8 medium bags throughout the experiment. Interestingly, the heterotrophic flagellate concentrations observed under the light microscope were much lower in the *Chlorococcum* sp. cultures compared to *Chlorella* sp. cultures, likely allowing the bacteria to thrive in the organic-rich potato juice medium.

As a result of observed dynamics between heterotrophic flagellates, bacteria and algae, growth curves in PJ medium data show a bimodal pattern, likely reflecting the initial growth of flagellates and bacteria followed by increase in algal concentrations. While these patterns have likely influenced automated growth rate calculations (possibly overestimating growth in potato juice), the final biomass concentration measurements reached in 1 % PJ medium are still valid and demonstrate that PJ-based media are indeed suitable for scaled-up algae cultivation.

3.5. Chemical analysis of algal biomass

3.5.1. Carotenoids

Mean concentrations of zeaxanthin, lutein and beta-carotene did not differ significantly between *Chlorella* sp. biomass cultivated in 1 % PJ

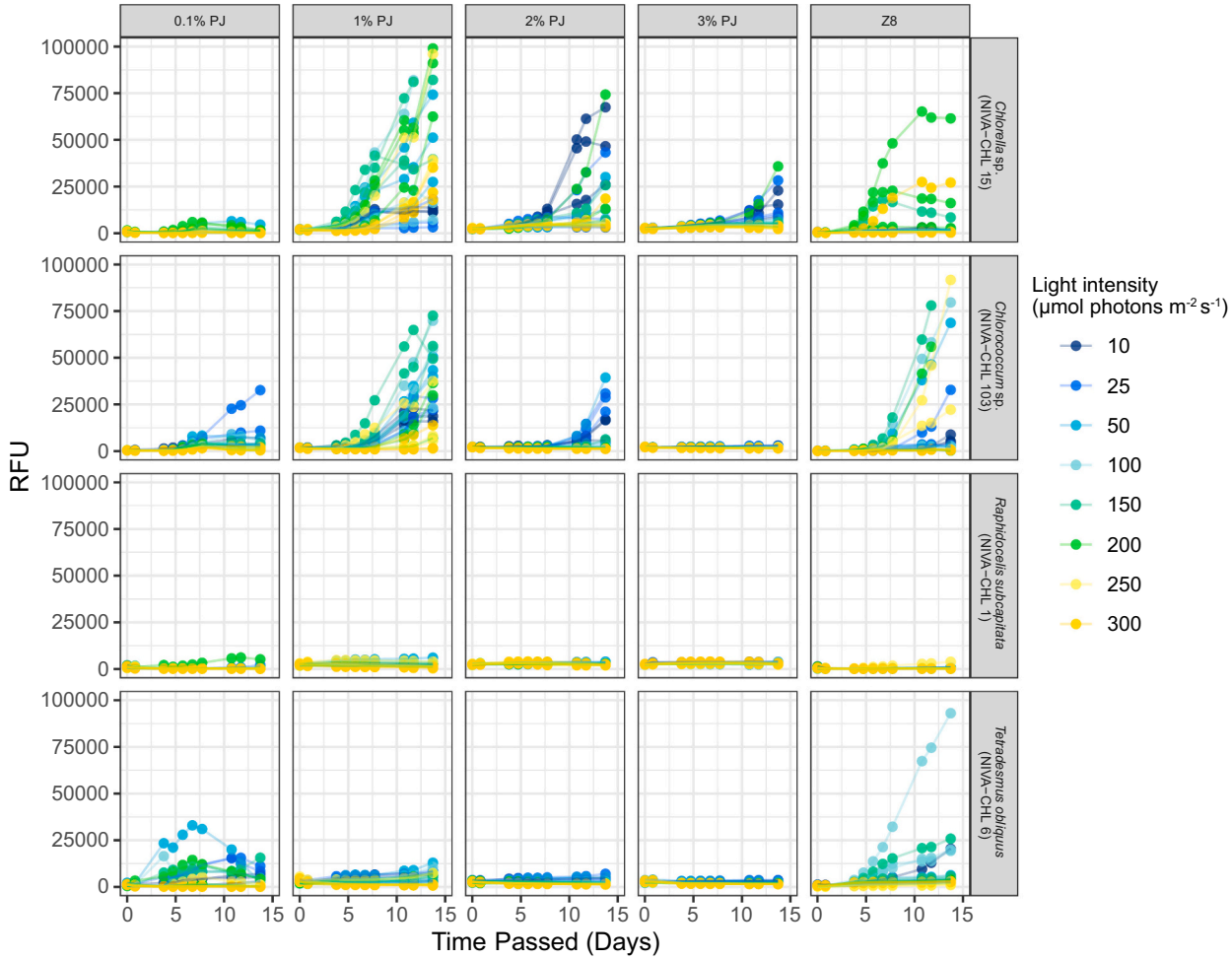

Fig. 3. Growth curves of strains in the optimization experiment, under different PJ medium formulations and increasing light intensities.

medium and biomass cultivated in Z8 medium (Fig. 7). On the other hand, astaxanthin was more concentrated in biomass from Z8 than in biomass from 1 % PJ medium. The most concentrated carotenoid in *Chlorella* biomass was lutein (454 mg kg^{-1} in Z8), followed by astaxanthin (178 mg kg^{-1} in Z8), with relatively low concentrations of beta carotene and zeaxanthin (<100 mg kg^{-1} in all treatments). These results are in line with available literature, which shows that that *Chlorella* is potentially a good source of lutein, especially for nutraceutical application [45]. However, lutein concentrations obtained from biomass in this study were order of magnitude lower than the highest reported yields in the literature that show values over 9000 mg kg^{-1} [45]. Lutein concentration is known to vary significantly among strains [46] and growth conditions, and lower light intensities and addition of nitrate were shown to increase its concentrations in *Chlorella vulgaris* [45]. Since no comparative differences were observed in lutein concentrations between Z8 and 1 % PJ media, this side stream should be further explored and optimized for circular and sustainable lutein accumulation in *Chlorella*.

In *Chlorococcum* sp., lutein had the highest mean concentration of all measured carotenoids, reaching 2295 mg kg^{-1} in Z8. Astaxanthin and beta-carotene concentrations were similar, both reaching more than 1000 mg kg^{-1} in Z8 medium. Interestingly, *Chlorococcum* sp. biomass grown in Z8 medium had one order of magnitude higher concentration of all analysed carotenoid pigments (except zeaxanthin) compared to *Chlorella* sp. biomass grown in 1 % PJ medium and in Z8 medium as well as *Chlorococcum* sp. grown in 1 % PJ medium. Clearly, the tested *Chlorococcum* strain shows potential for lutein accumulation, however only when grown autotrophically in the Z8 medium. Earlier studies have shown that, while *Chlorococcum* species are rich in lutein and other carotenoids, the concentrations of carotenoids decrease under organic carbon (glucose) enrichment, which can explain low carotenoid concentrations in organic carbon-rich 1 % PJ medium [47]. This explanation is supported by the growth data, which indicate that *Chlorococcum* treatments in 1 % PJ and Z8 were harvested in the stationary phase at very similar concentrations. While similar trend of mean decrease in carotenoids under 1 % PJ cultivation was observed in *Chlorella*, it was not statistically significant. It is possible that in case of *Chlorella*, the effect of organic carbon-rich medium was muted by difference in growth stage at the time of harvesting.

3.5.2. Fatty acid methyl esters (FAME)

The concentrations of saturated fatty acids (SFA) from the biomass cultivated in 1 %PJ and stock medium Z8 demonstrated typical profiles for green algae (Fig. 8) [48]. The SFAs concentration was slightly higher in *Chlorella* sp. grown in Z8 medium (22.14 %) compared to 1 % PJ medium (21.07 %). On the other hand, 1 % PJ medium-grown *Chlorococcum* sp. had higher SFA concentration (31.26 %) than Z8 medium-grown *Chlorococcum* sp. (26.86 %). Palmitic acid (C16:0), had highest contribution to SFA in *Chlorella* sp. biomass grown in Z8 medium (19.65 %) compared to PJ medium (17.82 %), but lower in *Chlorococcum* sp. biomass grown in Z8 medium (24.31 %) compared to PJ medium (29.15 %). Overall, *Chlorococcum* sp. biomass had higher concentration of Palmitic acid than *Chlorella* sp. biomass both in Z8 medium and in PJ medium. Other SFAs had minor contributions, usually lower than 1 % except for Stearic acid (C18:0) which was slightly more represented in both strains and under both treatments (up to 3 %).

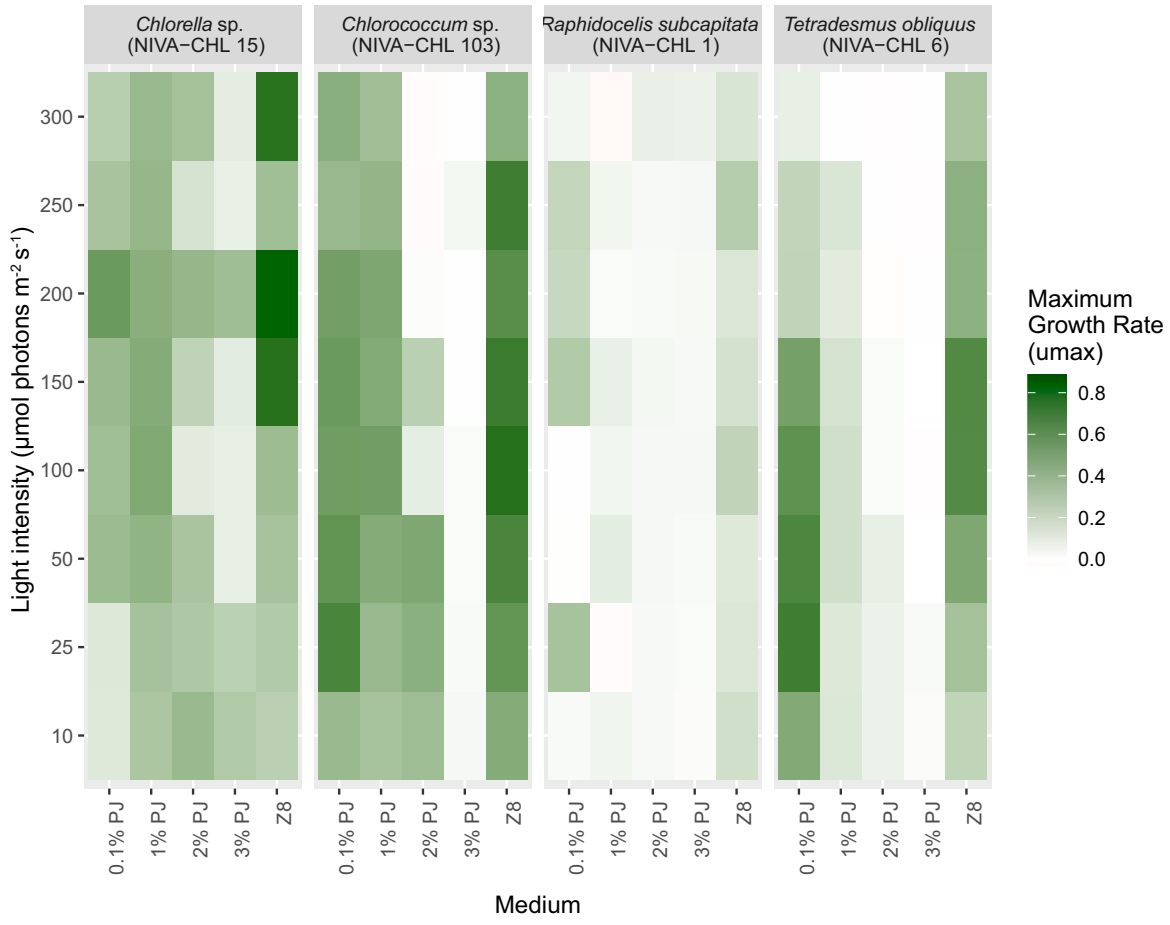

Fig. 4. Maximum growth rates measured for each strain during the optimization experiment in different PJ medium formulations and control medium Z8 under increasing light intensities. Only growth rate calculations with r2 > 0.7 are included.

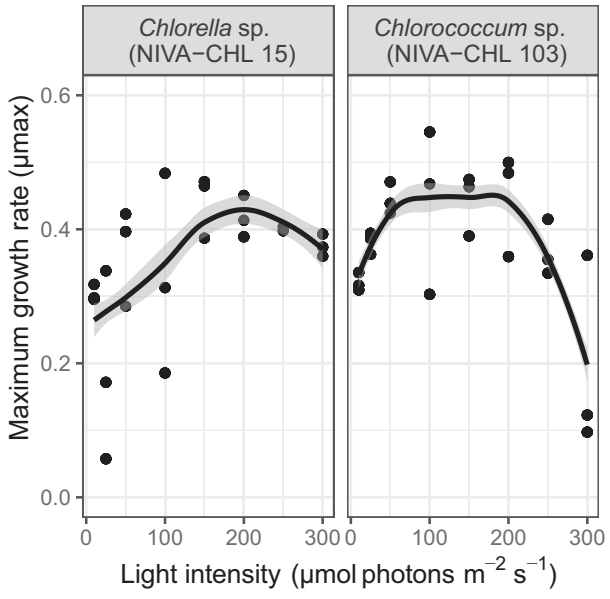

Fig. 5. Light optima of *Chlorella* sp. and *Chlorococcum* sp. when growing in 1 % PJ medium. Data points represent growth rates calculated from individual well-replicates. The smoothing curve was generated using *method = "loess"* (local polynomial regression fitting) in *geom_smooth* (R). Grey area represents a 95 % confidence interval of the mean. Only growth rate calculations with r2 > 0.7 are included.

Monounsaturated fatty acids (MUFA) had the highest overall contribution to FAME in *Chlorella* sp. and lowest in *Chlorococcum* sp. As was the case with SFAs, concentrations were similar between the tested media in *Chlorella*, being only slightly higher in Z8 medium (39.50 %) compared to PJ medium (36.81 %), and nearly two times higher in PJ medium (12.41 %) compared to Z8 medium (7.25 %) in *Chlorococcum*. In both strains, the main contributor to the MUFA content was Oleic acid (C18:1 n9c), with only minor contributions from other MUFAs.

Unlike SFA and MUFA, total contribution of polyunsaturated fatty acids (PUFA) was on average higher in *Chlorella* sp. growing in PJ medium (26.19 %) compared to Z8 medium (25.485), and higher in *Chlorococcum* sp. grown in Z8 medium (33.69 %) compared to PJ medium (29.14 %). Most concentrated PUFA species in both strains were essential Omega-3 α-Linolenic acid (C18:3 n3) and Linoleic acid (C28:2 n6c), accounting for 14–20 % and 7–11 %, respectively. Other, commercially important PUFAs such as EPA and DHA were not detected, as they are commonly low in *Chlorella* and *Chlorococcum* biomass [48,49].

Both strains exhibited fatty acid profiles characteristic for green algae, where Palmitic acid, Oleic acid, Linoleic acid and α-Linolenic acid account for >90 % of the fatty acid content [50]. While green algae are commonly poor in commercially important omega-3 fatty acids such as EPA and DHA, they are still recognized as a source of PUFA-biomass that can be used for a variety of applications, most notably in food and feed sectors and as feedstock in biofuel production [51]. Despite relatively conserved fatty acid profiles found in different microalgal groups, their concentrations can still be modified by various cultivation strategies. Studies on *Chlorella* have shown that fatty acid content can be

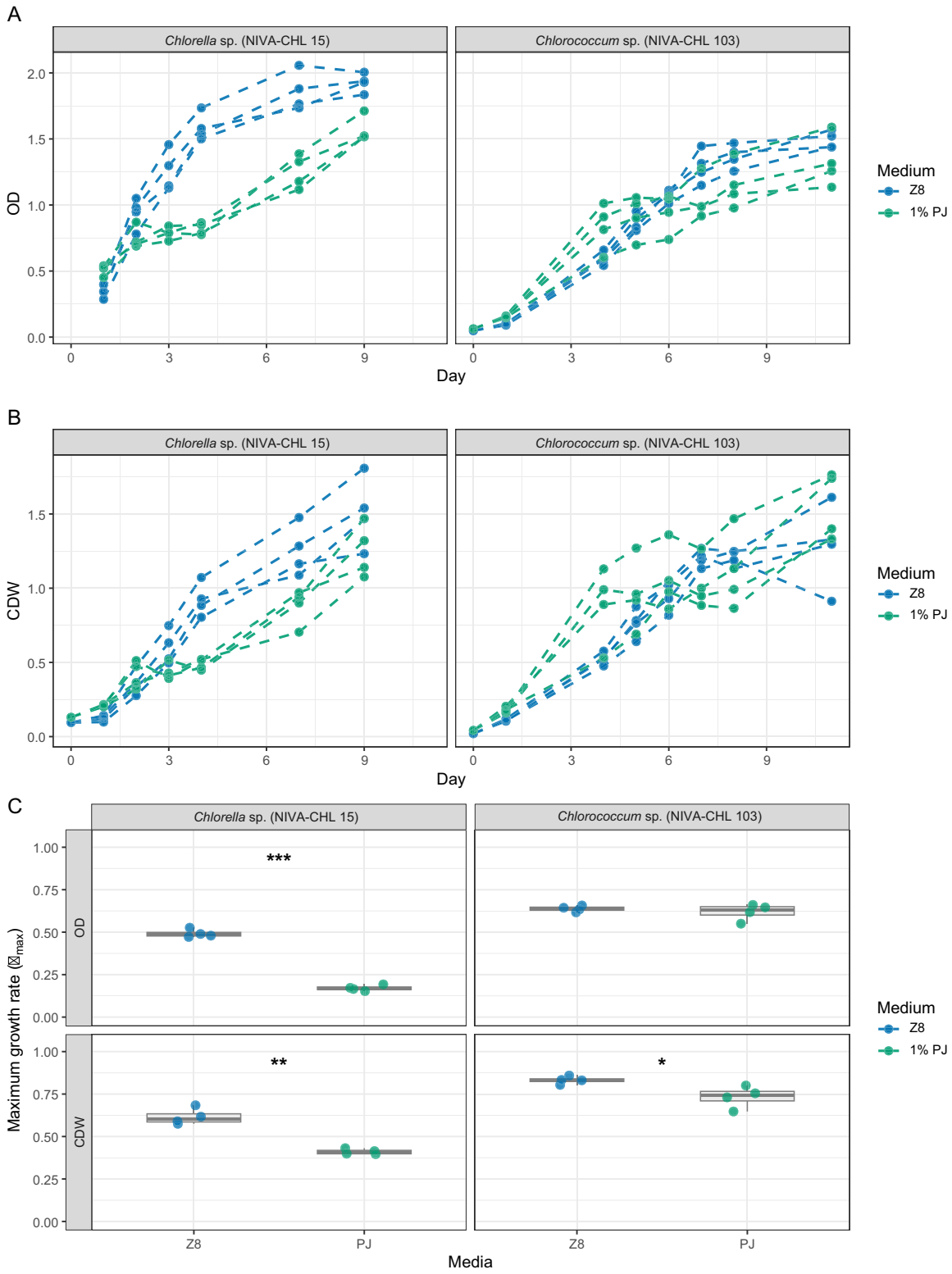

Fig. 6. Growth curves of the two strains used in the scale-up experiments: A) Optical density (OD) data; B) Cell dry weight (CDW) data; C) Comparison of growth rates calculated from OD and CDW data in 1 % PJ and Z8 medium. Asterisks indicate statistical significance of pairwise comparisons: $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***).

modulated by mixotrophic cultivation as well as other parameters such as light period, light intensity, organic carbon source and nutrient limitation [14,17,40,52]. In this work, cultivation in 1 % PJ was linked with decreased content of SFA and MUFA in *Chlorella* and increased concentrations of SFA and MUFA in *Chlorococcum*, with PUFA concentration of *Chlorococcum* decreasing in 1 % PJ medium. It is therefore clear that while cultivation in 1 % PJ does modulate FAME content, there are no obvious patterns detected. More research and dedicated growth optimization is needed to tailor the cultivation process in PJ-based media for the production of specific fatty acids.

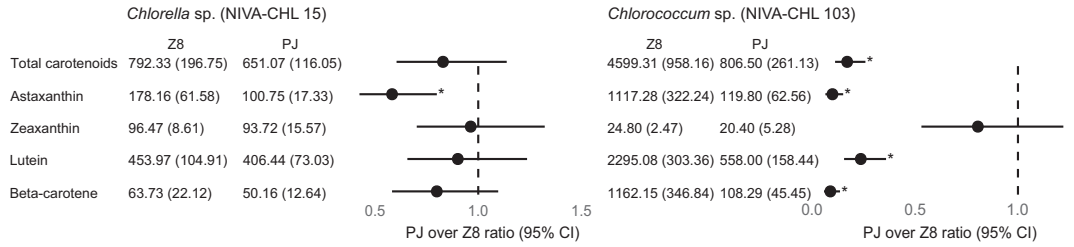

Fig. 7. Carotenoid pigment concentrations (mg kg^{-1}) in *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA-CHL 103) biomass obtained after growth in stock medium Z8 and 1 % PJ medium. Values indicate mean of 4 replicates with standard deviation. Astaxanthin concentration includes all-E, 9Z and 13Z isomers. Tree plots show proportional contrasts (PJ: Z8) derived from linear mixed-effects models fitted to log-transformed concentrations. Values represent geometric mean ratios, where values >1 indicate higher concentrations in PJ and values <1 indicate higher concentrations in Z8. Asterisks (*) denote statistically significant differences after Benjamini–Hochberg adjustment ($p < 0.05$).

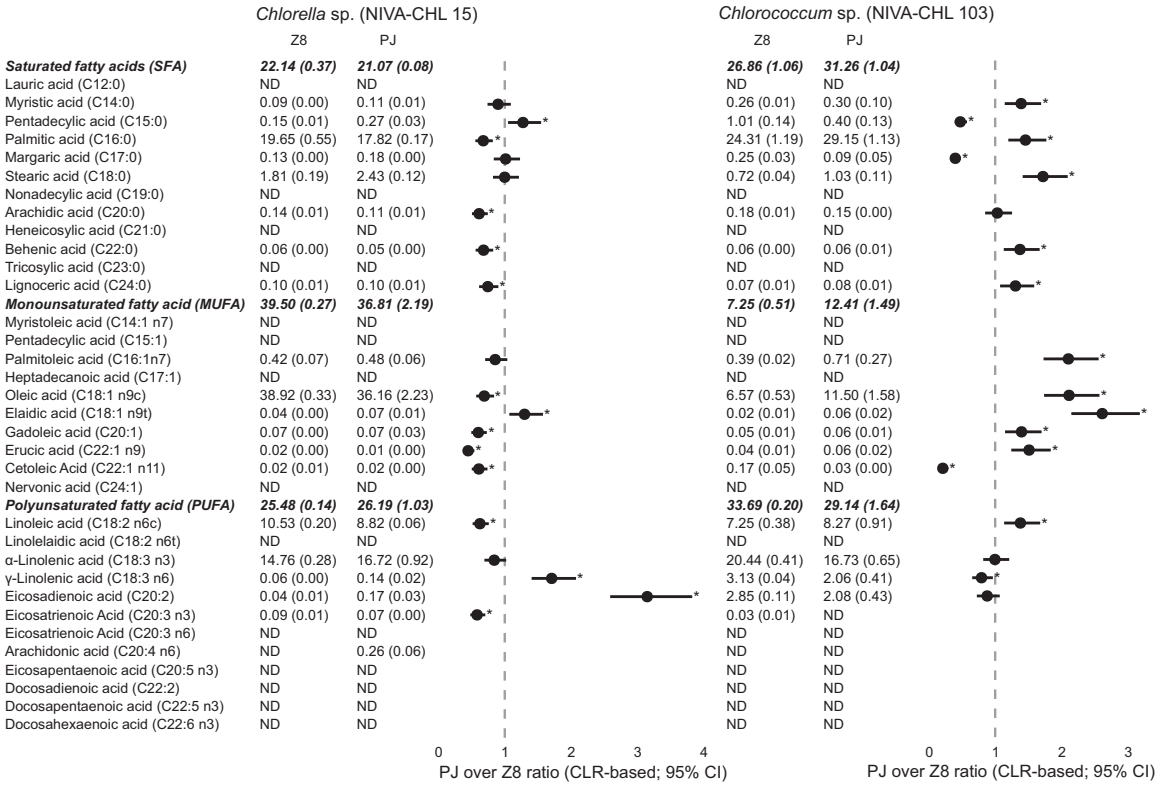

Fig. 8. Fatty acid concentrations (% of total fatty acids) in *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA-CHL 103) biomass obtained after growth in stock medium Z8 and 1 % PJ medium. Values indicate mean of 4 replicates with standard deviation. Tree plots show proportional contrasts (PJ: Z8) estimated from linear models fitted to centered log-ratio (CLR) transformed data. Values represent multiplicative changes in composition, where values >1 indicate higher proportions in PJ and values <1 indicate higher proportions in Z8. Asterisks (*) denote statistically significant differences after Benjamini–Hochberg adjustment ($p < 0.05$). ND = Not detected.

3.5.3. Amino acids

Amino acid concentrations measured in the collected biomass were generally in line with previous studies on microalgae including *Chlorella* [28,53]. The total of 17 amino acids were identified and analysed in the obtained microalgal biomass, with concentrations differing both among the strains and between the treatments (Fig. 9). Among these, eight out of nine essential amino acids were detected in the biomass of both strains, with exception of Tryptophan (Trp). *Chlorella* biomass grown in 1 % PJ medium had significantly higher concentrations of nearly all detected amino acids except for Arginine and Glutamic acid, and the concentration of total essential amino acids was significantly higher in *Chlorella* from 1 % PJ medium (77.15 g kg^{-1}) compared to biomass grown in Z8 medium (48.5 g kg^{-1}). The reason for strong increase in amino-acid content is unclear and it could be related to a variety of factors. For example, amino acids were shown to accumulate in *Chlorella* cells as a response to nitrogen limitation or cadmium toxicity [54]. Even though nitrogen limitation was not assessed during the scale-up experiment, the fact that 1 % PJ cultures were still in the late exponential phase at the time of harvesting indicate that they were not nitrogen limited. It is more plausible that differences in amino acid content reflected the physiological state of biomass from Z8 and 1 % PJ treatments, as it was shown that amino acid content is related to biomass productivity [55]. This may explain higher content in 1 % PJ biomass that was harvested in late exponential growth phase compared to Z8 biomass that was harvested in the stationary phase. While all amino-acid concentrations were significantly different among *Chlorella* sp. biomass cultivated in Z8 medium and 1 % PJ medium, the majority of amino acids had statistically same concentrations in *Chlorococcum* sp. cultivated in Z8 medium and 1 % PJ medium. Only four amino acids had significantly different concentrations between the two treatments in

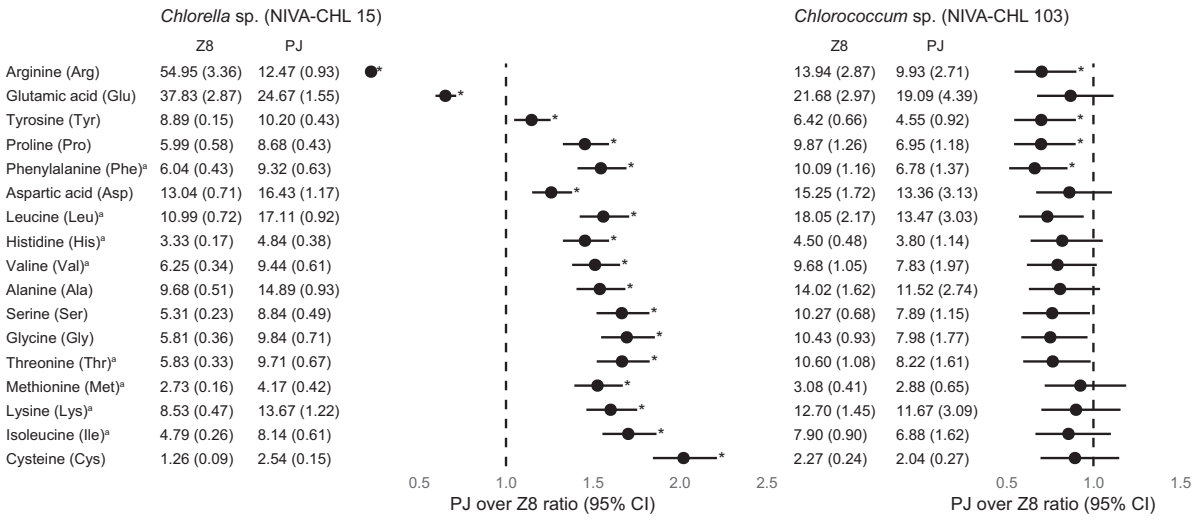

Fig. 9. Amino acid concentrations (g kg^{-1}) in *Chlorella* sp. (NIVA-CHL 15) and *Chlorococcum* sp. (NIVA-CHL 103) biomass obtained after growth in stock medium Z8 and 1 % PJ medium. Essential amino-acids are marked with ([a]) Values indicate mean of 4 replicates with standard deviation. Tree plots show proportional contrasts (PJ: Z8) derived from linear mixed-effects models fitted to log-transformed concentrations. Values represent geometric mean ratios, where values >1 indicate higher concentrations in PJ and values <1 indicate higher concentrations in Z8. Asterisks (*) denote statistically significant differences after Benjamini–Hochberg adjustment ($p < 0.05$).

Chlorococcum sp. (Phenylalanine, Proline, Serine and Tyrosine), all of which were more concentrated in Z8 medium-grown biomass. Overall, the concentrations of essential amino acids in *Chlorococcum* sp. were higher in biomass grown in Z8 medium (75.85 g kg^{-1}) compared to PJ medium (61.53 g kg^{-1}). In case of *Chlorococcum*, differences in amino acid concentrations were much less pronounced compared to *Chlorella*. This may also be related to the physiological state of the cultures which were both harvested in a stationary growth phase.

3.6. Potato juice as algae cultivation medium

The results of our study demonstrate that potato juice is a suitable substrate for scaled-up cultivation of *Chlorella* sp. and *Chlorococcum* sp., representing a promising side stream for commercial microalgae cultivation. This is in line with previous studies showing that microalgae can be grown at large scale in a range of plant-based agro-industrial side streams [15,56], including those originating from potato industry [19,31,35,57]. While previous studies employed different pre-treatment approaches such as hydrolyzation and anaerobic digestion to render the potato juice more suitable for microalgae cultivation, we used an approach with relatively simple pre-treatment techniques (sedimentation, decanting, dilution and filtration) in order to provide a cost-effective cultivation strategy.

Chemical analysis of potato juice performed in this study confirmed that potato juice is highly suitable for algae cultivation due to high concentrations of nitrogen, phosphorus and organic carbon, as well as selected micronutrients. Even the nutrient concentrations in 1 % PJ medium were comparable to the standard Z8 medium and were sufficient for scaled-up cultivation of algae in our experiment. Furthermore, during the screening, best growing strains showed higher growth rates in 1 % potato juice in dH_2O compared to 1 % potato juice in Z8 medium. This reveals that algae were not limited in micronutrients when growing in the potato juice.

A significant challenge for phototrophic microalgae cultivation in a potato juice is posed by its very low transparency, which was likely related both to suspended solids and to various humic and tannic acids present in the potato juice that were not removable by sedimentation and filtration steps [23]. To overcome this issue, we have tested different dilution steps and light intensities, balancing the trade-off between nutrient availability in diluted potato juice and sufficient light intensity for phototrophic growth. In addition, these compounds were found to be very stable and resistant to microalgal and microbial activity, as the dark brown colour of potato juice remained even after harvesting of algae. Finding an efficient and sustainable pre-treatment strategy that would decrease the concentration of humic and tannic acids and improve transparency of the juice would likely enable higher nutrient concentrations and more efficient phototrophic growth. While the dilution approach used in this study was successful, water recycling should be explored for more sustainable dilution of potato juice at industrial scale [58].

Another important feature of potato juice is a high concentration of organic substances, primarily starch and proteins, which makes it highly suitable for cultivation of both heterotrophic and mixotrophic organisms. As highlighted in this study, high organic content also increases the contamination risk of bacteria and heterotrophic protists during scaled-up phototrophic cultivation. Contamination is a well-known challenge in large-scale algae cultivation, especially when using side streams and similar substrates [4,18]. On the other hand, the potato juice has high potential for mixotrophic or heterotrophic cultivation of algae, for example *Chlorella*, which was likely favoured in the screening design used in this study due to its capacity for utilizing organic carbon. However, purely heterotrophic cultivation in potato juice would require axenic microalgal strains and more substantial pre-treatment of potato juice that could eliminate any bacterial and protist contamination from the system [43]. This means that auto- or mixotrophic cultivation may prove to be the most cost-effective for valorisation of potato juice and creation of new value from this side stream.

While this research shows promising potential for valorization of potato juice using microalgae biotechnology, the implementation of this technology should consider the regulatory aspects regarding the final use of algal biomass. This concerns both the cultivation of microalgae in waste or a side stream, as well as specific regulation concerning the use of algae for food or feed [59]. For example, while several species of *Chlorella* are food approved in the EU under Novel Food Regulation (EU 2017/2470) and thus suitable for human consumption, this is still not the case for *Chlorococcum*. Furthermore, algae grown on waste or side streams are not permitted for human consumption but may be suitable for animal feed or agriculture applications if region-specific regulatory and safety requirements are met [59,60]. In this sense, potato juice-grown algal biomass may find its application in feed and agriculture as well as biogas or biofuel sectors.

4. Conclusions

Nutrient-rich agro-industrial side streams represent a promising substrate for industrial microalgae cultivation and creation of added value products from waste. Still, an efficient methodology for screening and growth optimization is essential for identifying algal strains that can grow in side streams and for upscaling of microalgae cultivation. This study demonstrated that potato juice, a by-product of potato starch industry is a suitable substrate for scaled-up cultivation of commercially interesting algae *Chlorella* sp. and *Chlorococcum* sp., yielding biomass growth comparable to that in the standard growth media. Along with comparable biomass yields, biomass grown in potato juice showed biochemical profiles of carotenoids, fatty-acids and amino-acids that were similar to the biomass growing in control medium. Specific challenges related to microalgae cultivation in potato juice have also been identified, mainly related to potential contamination due to high organic content of the side stream, and need for sustainable pre-treatment method to account for high turbidity of potato juice.

CRediT authorship contribution statement

Luka Šupraha: Writing – original draft, Methodology, Investigation, Conceptualization. **Margarida Costa:** Writing – review & editing, Methodology, Investigation. **Trine Dale:** Writing – review & editing, Project administration, Conceptualization. **Minna Kahala:** Writing – review & editing, Resources, Project administration. **Vesa Joutsjoki:** Writing – review & editing, Resources. **Anne Pihlanto:** Resources, Project administration, Funding acquisition. **Ethan Wood:** Writing – review & editing, Investigation. **Yan Li:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

We have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2025.104328.

Data availability

Data will be made available on request.

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