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Improving the content of high value compounds in Nordic *Desmodesmus* microalgal strains

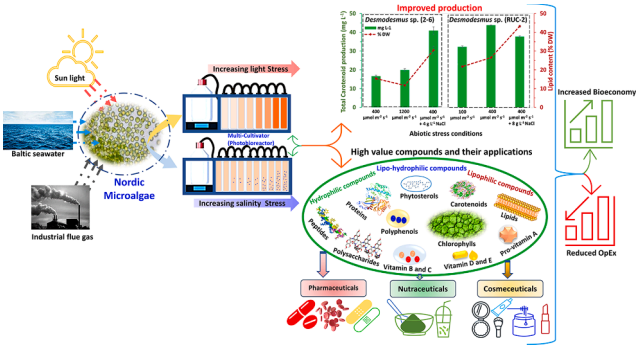
Sanjeet Mehariya [a], Martin Plöhn [a], Antonio Leon-Vaz [a,b], Alok Patel [c], Christiane Funk [a,*]

[a] Department of Chemistry, Umeå University, Umeå, Sweden
[b] Laboratory of Biochemistry, University of Huelva, Huelva, Spain
[c] Biochemical Process Engineering, Division of Chemical Engineering, Luleå University of Technology, Luleå, Sweden

HIGHLIGHTS

- Nordic microalgae produce compounds of high value when exposed to abiotic stress.
- Light stress stimulates carotenoid production up to 44 mg L^{-1} in *Desmodesmus* sp.
- Combined light and salt stress enhanced the lipid content by 43% of DW.
- Nordic microalgae grown in various wastewaters can produce carotenoids and lipids.

GRAPHICAL ABSTRACT



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ABSTRACT

Nordic *Desmodesmus* microalgal strains (2–6) and (RUC-2) were exposed to abiotic stress (light and salt) to enhance lipids and carotenoids. The biomass output of both strains increased by more than 50% during light stress of 800 μmol m^{-2} s^{-1} compared to control light. The biomass of *Desmodesmus* sp. (2–6) contained most lipids (15% of dry weight) and total carotenoids (16.6 mg g^{-1}) when grown at moderate light stress (400 μmol m^{-2} s^{-1}), which further could be enhanced up to 2.5-fold by salinity stress. *Desmodesmus* sp. (RUC-2) exhibited maximal lipid (26.5%) and carotenoid (43.8 mg L^{-1}) content at light intensities of 400 and 100 μmol m^{-2} s^{-1}, respectively. Salinity stress stimulated lipid accumulation by 39%. Nordic *Desmodesmus* strains therefore are not only able to tolerate stress conditions, but their biomass considerably improves under stress. These strains have high potential to be used in algal bio-factories on low-cost medium like Baltic seawater.

1. Introduction

In the presence of light, microalgae use carbon dioxide (CO_2) to produce biomass and compounds which have industrial applications in the food-, feed- and fuel-sectors. Microalgal based biotechnology platforms therefore support the United Nations "Sustainable Development Goals" (SDGs) (Kholany et al., 2022). Microalgae have a fast growth rate, and their CO_2 sequestration is up to 50% higher than terrestrial

* Corresponding author.
E-mail address: Christiane.Funk@umu.se (C. Funk).

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crops (Goswami et al., 2022a; Park et al., 2022). In addition, microalgae cultivation can be carried out on non-arable lands and therefore does not compete with agriculture or forestry. During CO_2 sequestration, microalgae synthesise various biomolecules (Mehariya et al., 2021) with high market demand due to their industrial applications (Zhou et al., 2022). After the extraction of high value compounds, the leftover/residual biomass can be further converted into biofuel through pyrolysis (Huang et al., 2022). However, the microalgal based biotechnologies have not been extensively commercialized due to the high capital and operational expenditure (CapEx and OpEx) of up- and downstream processing (Kumar et al., 2020). To decrease the production costs microalgal cultivations can be performed in cheap medium such as sea- or wastewater (Li et al., 2022). At the same time biomass production and composition has to be optimised (Goswami et al., 2022a). Exposure to biotic or abiotic stress has been shown to increase the carotenoid and/or lipid content of microalgal cells (Paliwal et al., 2017). However, stress may also lead to relatively lower biomass production, subsequently causing lower productivity of intracellular molecules. To optimize the biomass and biomolecule productivity, "ideal" stress conditions need to be investigated for individual algal strains of interest to enhance the production of intracellular molecules.

Algal growth is well known to be dependent on abiotic factors like light, temperature and nutrient availability. A nitrogen (organic and inorganic) source is essential for algal metabolism, and growth (Kumar and Singh, 2019). As proteins, chlorophylls and nucleic acid contain nitrogen, N-starvation therefore stimulates lipid and carbohydrate production in the algal cell (Latsos et al., 2020). Light stress, on the other hand, is considered to improve carotenoid production to protect the cells from photoinhibition (Mehariya et al., 2020; Varela et al., 2015). Nordic algae are well adapted to low temperatures (Ferro et al., 2018b), however, the bright days in combination with relatively moderate temperatures, as commonly experienced in Scandinavia, create stress conditions, potentially leading to improved biomass content. Recently, Nzayisenga et al. (2020) explored the effect of light stress (50–300 μmol m^{-2} s^{-1}) in various Nordic microalgal species isolated from Sweden. Among the five tested microalgae, *Desmodesmus* sp. improved its biomass production 3.5-fold during growth at moderate light stress (300 μmol m^{-2} s^{-1}) compared to normal growth conditions (50 μmol m^{-2} s^{-1}). Biomass and lipid yields were increased by 1.4-and 1.3-fold, respectively in chemostatic cultivation of *Ettlia* sp. under light stress (1500 μmol m^{-2} s^{-1}) as compared lower light stress (1500 μmol m^{-2} s^{-1}) (Seo et al., 2017). Recently, the effect of different light stress (635–2300 μmol m^{-2} s^{-1}) was evaluated while cultivating *Arthrospira platensis*: Improved biomass (0.62 g L^{-1} d^{-1}) production with a phycocyanin content of ~ 20% was obtained at 2300 μmol m^{-2} s^{-1} during semi-continuous cultivation (Chaiklahan et al., 2022). The optimal light intensity for growth and biomolecule production therefore varies between microalgal species (Maltsev et al., 2021).

Salinity stress is considered as another feasible approach to enhance biomass, carotenoid and lipid production. This approach has the advantage of allowing the use of seawater for large scale cultivation. Rocha et al. (2019) reported that salinity stress improves the lipids content in *Scenedesmus* sp. However, high salinity may reduce the biomass production and subsequently, the lipid production of the algae. Therefore, optimal salinities and light intensities need to be investigated for individual microalgal strains to demonstrate the possible use of sun light and seawater for microalgae cultivation and the production of valuable compounds.

In this study, the effects of light and salt stress were evaluated on two Nordic freshwater microalgal strains of the genus *Desmodesmus* to improve their biomass, carotenoid and lipid production. *Desmodesmus* strains have shown a wide range of biotechnological applications including resource recovery from various low-cost media, for example anaerobic digestion effluent (Huang et al., 2022; Li et al., 2022; Rugnini et al., 2018). Various species of *Desmodesmus* have been considered for the production of high value compounds including proteins, pigments, carbohydrates and lipids (Goswami et al., 2022a; Sun et al., 2022). The Nordic *Desmodesmus* strains (2–6) and (RUC-2) originate from Southern (2–6) and Northern (RUC-2) Sweden and their potential to grow on municipal wastewater (MWW) had been assessed (Ferro et al., 2018a). Their ability to grow in saline conditions would open the possibility to use other low-cost media (e.g. Baltic seawater) for cheap and sustainable cultivation, which also would allow their biomass to be used for food and feed. During the cultivation, growth performance and photosynthetic efficiency were monitored at alternate days, while net biomass, total carotenoid and lipid content were analysed quantitatively and qualitatively at the end of the experiments.

2. Materials and methods

2.1. Microalgal culture and chemicals

All constituents of the culture medium used for microalgae growth and chemicals for analysis were bought (Sigma-Aldrich, USA), unless detailed elsewhere. To ensure reproducible growth conditions, BG-11 culture medium was used containing $NaNO_3$ (1.50 g L^{-1}), K_2HPO_4 (40.0 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (75.0 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (36.0 mg L^{-1}), Citric acid (6.0 mg L^{-1}), Ammonium ferric citrate green (6.0 mg L^{-1}), $EDTANa_2$ (1.0 mg L^{-1}), Na_2CO_3 (20.0 mg L^{-1}) and 1 mL of trace metal solution. The trace metal solution contains: H_3BO_3 (2.86 g L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.81 g L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.22 g L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.39 g L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.08 g L^{-1}), $Co(NO_3)_2{\cdot}6H_2O$ (0.05 g L^{-1}) (Singh et al., 2020).

The Nordic algal strain *Desmodesmus* sp. (2–6) has been isolated from the fresh water lake Ringsjön in Southern Sweden, while *Desmodesmus* sp. (RUC-2) was isolated from Umeå River, Umeå, Northern Sweden (Ferro et al., 2018a). Both *Desmodesmus* sp. strains were maintained in liquid BG-11 medium at 50 μmol m^{-2} s^{-1} of light intensity and 18 ± 2 °C and routinely subcultured in fresh BG-11 medium under aseptic conditions. A pre-inoculum (100 mL) of each strain was grown in sterile conical flasks of 250 mL using BG-11 medium at pH 7.2 and 18 ± 2 °C. The culture was shaken at 120 rpm using an orbital shaker incubator with inbuilt illumination panel and maintained at 100 μmol m^{-2} s^{-1}.

During all experiments *Desmodesmus* sp. (2–6) and (RUC-2) were cultivated in an air lift photobioreactor (working volume of 80 mL) (Multi-Cultivator MC 1000-OD; PSI, Czech Republic) using BG-11 medium. The cultures were continuously bubbled with air mixed with 3% CO_2 at a constant flow rate of 50 mL min^{-1} at 18 ± 2 °C for 14 days. A one-week-old pre-culture of each strain was inoculated to sterile BG-11 medium to achieve an optical density (OD_{750}) of 0.1. To achieve light stress the algae were exposed for 14 days to continuous illumination at different intensities (100, 400, 800, and 1200 μmol m^{-2} s^{-1}) of white light, which were termed as mild, moderate, high and very high light stress, respectively. Salt stress was applied at 400 μmol m^{-2} s^{-1} with different NaCl (0, 2, 4, 8, 16 g L^{-1}) concentrations added to BG-11 medium, termed as without, low, mild, moderate and high salt stress, respectively. All stress experiments were performed in duplicate and growth was monitored for 14 days.

2.2. Optical density and photosynthetic efficiency

Microalgal growth measurements were carried out every alternate day by determining the optical density of the cultures at distinct wavelengths (OD_{530}, OD_{680}, and OD_{750}) using a T90+ UV–visible spectrophotometer (PG Instruments Limited, UK). Photosynthetic efficiency of the microalgal cells, i.e., quantum yield (Q_y) of Photosystem-II, was analysed using a compact pulse amplitude modulation (PAM) fluorimeter (AquaPen-C AP-C 100, PSI, Czech Republic). Before measuring Q_y, the cultures were diluted to OD_{680} of 0.5 to avoid overflow errors and incubated for 30 min in darkness for cell adaptation.

2.3. Biomass harvesting and dry weight estimation

After 14 days of growth the microalgal cultures were centrifuged for 10 min at 12,300 *g*. After washing two-times using Milli-Q water to avoid residual media components the pellets were lyophilized for 24 h, their dry weight (DW) were evaluated gravimetrically (Goswami et al., 2022b).

2.4. Fourier transform infrared spectroscopy (FTIR) analysis

Freeze-dried biomass of both *Desmodesmus* sp. strains was used for FTIR analyses as described by Gorzsás and Sundberg, (2014). The FTIR grade KBr was mixed with freeze-dried biomass and analysed by diffuse reflectance infrared FT-spectroscopy (DRIFTS) as described previously (Plöhn et al., 2022).

2.5. Lipid extraction and estimation

Freeze-dried biomass (150 mg) was milled manually using mortar and pestle and mixed with methanol:chloroform (1:2 v v^{-1}) (5 mL) and stirred for 2 h to attain complete recovery of lipids. Then distilled water (2.5 mL) was added and mixed properly before centrifugation for phase separation. The lower layer of lipid containing solvent was transferred to pre-weighed fresh glass vials. Lipid content was analysed gravimetrically after complete solvent evaporation as described earlier (Patel et al., 2022b).

2.6. Fatty acids profiling

After lipid extraction, transesterification was carried out for fatty acid (FA) analysis by gas chromatography-mass spectrometry (GC–MS). Transesterification of lipids was carried out in ace glass pressure vials with slight modifications as described by Van Wychen and Laurens, (2013). Extracted lipids were dissolved in methanol:chloroform: (1:2 v v^{-1}) mixture and catalysed by HCl (0.6 M) in methanol. After proper mixing the vials were kept in a water bath at 90 ± 2 °C for 1 h. After incubation the vials were kept at ambient conditions for extraction of fatty acid methyl esters (FAME) using *n*-hexane as extraction solvent. The FA content was measured by Clarus SQ 8C GC–MS (PerkinElmer, Inc., USA), which was equipped with a capillary column (Elite -FFAP; PerkinElmer, Inc., USA). Operative conditions were used for GC–MS analysis as described previously (Patel et al., 2022a).

2.7. Pigment extraction and analysis

For pigment extraction, 5 mg of freeze-dried biomass was added to MN bead tube Type A (Macherey Nagel, Düren, Germany), then 1 mL of methanol was added. The cells were disrupted by agitation with MN beads for 3 cycles of 2 min. After that, the pigments were extracted for 15 min using 1 mL of methanol at 70 ℃ and the homogenate centrifuged at 14,000 *g* for 5 min. The extraction process was repeated twice and the mix of the two supernatants was further analysed through HPLC. Quantification of individual carotenoids was performed in a Merck Hitachi HPLC equipped with UV–vis DAD detector as described by Rengel et al. (2022), with a column flow rate that was maintained at 1 mL min^{-1} using a RP-18 column. Carotenoid and chlorophyll detection was performed at 450 nm and the pigments were quantified using their correspondent standards.

3. Results and discussion

3.1. Effect of abiotic stress on growth and photosynthetic efficiency

Photoautotrophic growth of microalgae varies on the availability of nutrients, inorganic carbon (CO_2) and light. Optimal light intensity improves the biomass production and the accumulation of intracellular biomolecules (Patel et al., 2019). In this study, two Nordic freshwater microalgal strains belonging to the genus *Desmodesmus* were explored (Ferro et al., 2018a) with the aim to improve their biomass production by exposing them to light stress (Fig. 1) and additional salt stress (Fig. 2). Compared to mild light stress (100 μmol m^{-2} s^{-1}), the cell proliferation rate significantly increased during growth in moderate light (400 μmol m^{-2} s^{-1}). High and very high light stress did not improve the growth rate of *Desmodesmus* sp. (2–6) (Fig. 1a), however, slight improvements were observed in *Desmodesmus* sp. (RUC-2) (Fig. 1b). The maximal absorbance of *Desmodesmus* sp. (RUC-2) at very high light stress (1200 μmol m^{-2} s^{-1}) increased by 28% in comparison to the control (100 μmol m^{-2} s^{-1}). *Desmodesmus* sp. (2–6) entered the stationary growth phase at day 8 of cultivation in continuous light, while *Desmodesmus* sp. (RUC-2) stayed in the logarithmic growth phase much longer, when grown in mild light stress (100 μmol m^{-2} s^{-1}). Also other algal species are known to increase their growth rate when exposed to high light, e.g. *Chlorella protothecoides* (UTEX-256) (Patel et al., 2019).

Despite the improved algal growth, the photosynthetic efficiencies (quantum yields of Photosystem-II (Q_y)) of both *Desmodesmus* strains decreased during cultivation at higher light intensity due to photoinhibition (see supplementary material). In comparison to growth at mild light stress (100 μmol m^{-2} s^{-1},), the Q_y of *Desmodesmus* sp. (2–6) decreased by 14% (Q_y 0.59), 23% (Q_y 0.53) and) 26% (Q_y 0.51) during 14 days of growth at 400, 800 and 1200 μmol m^{-2} s^{-1}, respectively. Compared to the control, Q_y of *Desmodesmus* sp. (RUC-2) was impaired by 15–19% after 14 days of growth in moderate to very high light intensity. When stressed with 800 or 1200 μmol m^{-2} s^{-1}, the Q_y of *Desmodesmus* sp. (2–6) dropped at day 2, and then increased to an initial level before constantly decreasing (see supplementary material). This dip could be explained by damage and repair of the photosynthetic apparatus at high light (Solovchenko et al., 2013). A comparable observation was reported by Schipper et al. (2021) and related to photooxidation during the first 24 h of cultivation, while the culture was able to recover within 72 h. Contrary, *Desmodesmus* sp. (RUC-2) showed increased Q_y of PS-II at day 4, which then gradually decreased (see supplementary material).

Furthermore, the biomass production of both strains was significantly enhanced during growth in increased light intensities. Maximal biomass production was 3.8 and 5.2 g L^{-1}, respectively, for *Desmodesmus* sp. (2–6) and (RUC-2), during cultivation at 800 μmol m^{-2} s^{-1}; in comparison to 100 μmol m^{-2} s^{-1} the biomass production was enhanced by 1.4- and 1.5-fold, respectively (Fig. 1c). *Desmodesmus* sp. (RUC-2) produced 21–30% more biomass compared to *Desmodesmus* sp. (2–6) at the various light intensities. Similarly, rising the light intensity from 50 to 300 μmol m^{-2} s^{-1} was observed to optimize biomass production in *Desmodesmus* sp. (Nzayisenga et al., 2020). The biomass of a thermotolerant *Desmodesmus* sp. strain F51 was improved 1.6-fold when light intensified from 150 to 750 μmol m^{-2} s^{-1} (Xie et al., 2013).

Based on these light stress experiments on Nordic *Desmodesmus* strains, moderate light stress (400 μmol m^{-2} s^{-1}) was considered optimal for biomass production of (2–6) and (RUC-2) and was used in further experiments. A moderate light stress at 500 μmol m^{-2} s^{-1} also seems to be favourable for other microalgae, e.g. *Scenedesmus quadricauda* (Fettah et al., 2022).

To investigate the possibility to cultivate the *Desmodesmus* strains in a broader range of saline wastewaters (Yang et al., 2022) or even Baltic seawater with 2-3‰ salinity in the Bay of Bothnia, around Umeå (Sandberg et al., 2004), the effect of salt on growth and photosynthetic efficiency was tested. *Desmodesmus* sp. (2–6) and (RUC-2) were cultivated with different concentrations of NaCl ranging from 0 to 16 g L^{-1} (Fig. 2) at their optimal light stress (400 μmol m^{-2} s^{-1}). Increasing NaCl concentrations significantly decreased the growth rate of *Desmodesmus* sp. (2–6). In contrast, *Desmodesmus* sp. (RUC-2) showed a higher salt tolerance: low (2 g L^{-1}) and mild (4 g L^{-1}) NaCl concentrations enhanced the cultures growth rate, while moderate and high NaCl stress inhibited the growth compared to the control (no salt). Similar results

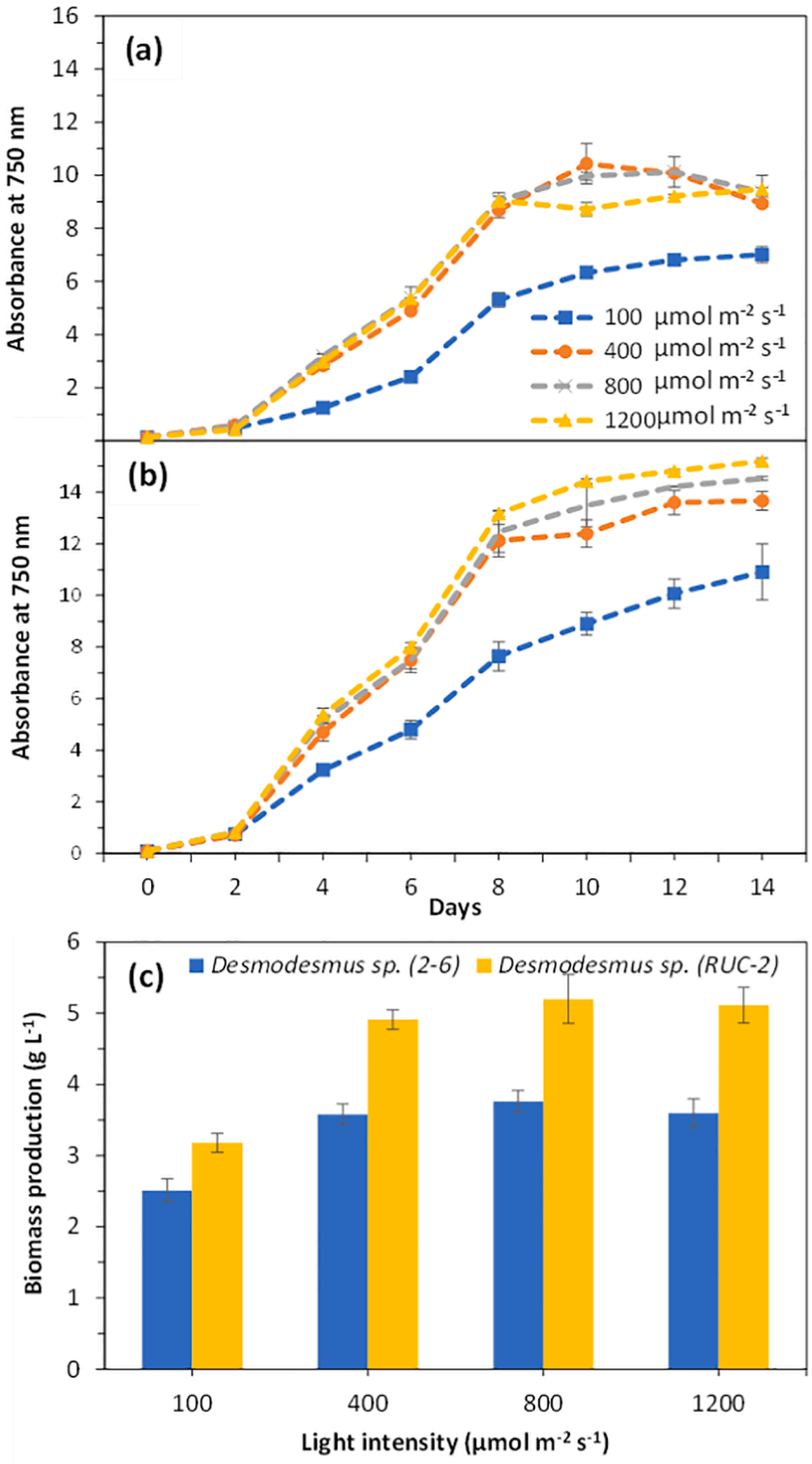

Fig. 1. Growth curve (based on OD_{750}) and biomass production in dependence to light intensity. Growth of (a) *Desmodesmus* sp. (2–6), (b) *Desmodesmus* sp. (RUC-2) and (c) biomass production of both strains after 14 days of growth.

were reported for *S. quadricauda* (Sq19), *S. dimorphus* (Sd12) and *Chlorella* sp. (Chl16) cultures, which displayed slightly improved growth at salt concentrations of 2.4 g L^{-1} in BG-11 medium (Gour et al., 2020). Yun et al. (2019) observed that 1.7 g L^{-1} NaCl enhanced the biomass production of *Chlorella vulgaris* YH703, while higher NaCl concentrations had a negative effect. The quantum yield of Nordic *Desmodesmus* strains increased in the presence of salt during the logarithmic growth phase and then decreased (see supplementary material). High concentrations of salt ions can cause imbalance in cell homeostasis, increasing the amount of reactive oxygen species (ROS) and, consequently, the reduction of cell development and inhibition of the growth (Ji et al., 2018).

Biomass production of *Desmodesmus* sp. (2–6) showed only small changes due to salt stress, increasing concentrations of NaCl from 2, 4

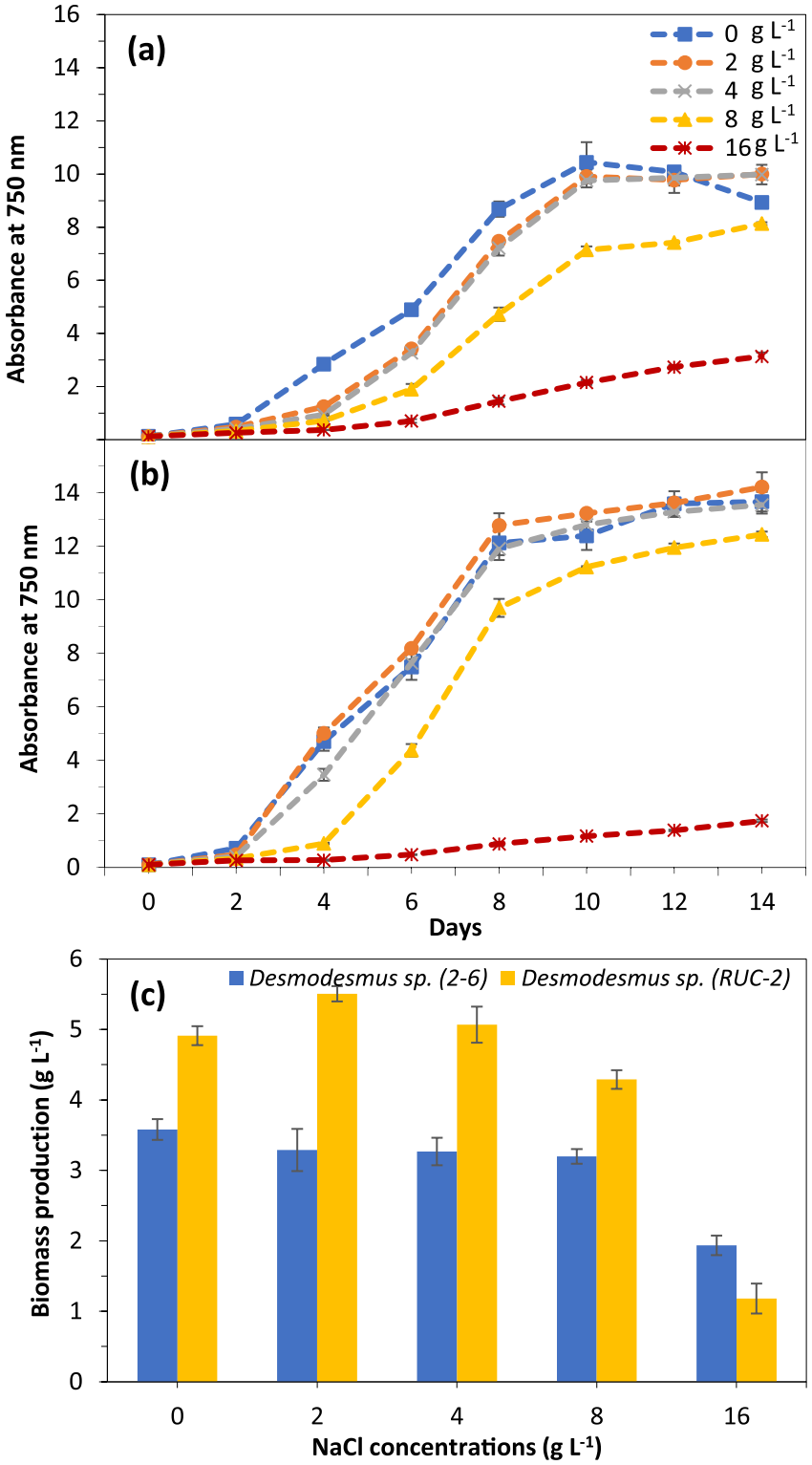

Fig. 2. Growth curve (based on OD_{750}) and biomass production in dependence to increased salt concentrations. Growth of (a) *Desmodesmus* sp. (2–6), (b) *Desmodesmus* sp. (RUC-2) and (c) biomass production of both strains after 14 days of growth.

and 8 g L^{-1} reduced the biomass production only by 5%, 6% and 8%, respectively. High salt stress (16 g L^{-1}), however, decreased the biomass production of *Desmodesmus* sp. (2–6) by 44%. Also in *Scenedesmus* sp. CCNM 1077 a gradual diminishing of the biomass production was observed at increasing NaCl concentrations (Pancha et al., 2015). Ji et al. (2018) observed that NaCl stress inhibited the growth of *Scenedesmus obliquus* XJ002. In contrast, the Nordic *Desmodesmus* sp. strain (RUC-2) was able to produce more biomass (5.5 g L^{-1}) under salt stress (2 g L^{-1} NaCl) (Fig. 2c). Further increases in NaCl concentration gradually decreased its biomass production. *Desmodesmus* sp. (RUC-2) was capable to produce 1.7-fold higher biomass as compared to *Desmodesmus* sp. (2–6) under salt stress (2 g L^{-1} NaCl) and therefore seems to be more robust. *Chlorella vulgaris* YH703 was able to grow faster during moderate salt stress (1.7 g L^{-1} NaCl) with enhanced biomass production. Yun et al.

(2019) observed that *Chlorella vulgaris* YH703 is able to tolerate oxidative stress caused by high salinity.

3.2. Effect of abiotic stress on lipid production

The microalgal lipid content can vary due to abiotic stress factors, including light, nutrients, salinity, temperature, trace metals, and other possible stress inducers (Rugnini et al., 2018). To investigate if the moderate light stress in combination with salt stress induced the production of lipids, *Desmodesmus* sp. (2–6) and (RUC-2) were grown in different light intensities and/or NaCl concentrations and the lipid content of their biomass was analysed (Fig. 3). Moderate light stress of 400 μmol m^{-2} s^{-1} significantly improved the lipid content in the biomass of both *Desmodesmus* strains (Fig. 3a), which was 15.3% DW in the *Desmodesmus* sp. (2–6) and 26.5% DW in the *Desmodesmus* sp. (RUC-2) biomass. *Desmodesmus* sp. (RUC-2) exhibited 1.7-fold higher lipid content compared to *Desmodesmus* sp. (2–6) in these conditions. Further increase in light intensity diminished the lipid content in *Desmodesmus* sp. (2–6), while *Desmodesmus* sp. (RUC-2) did not show any considerable change in lipid content (Fig. 3). The thermotolerant *Desmodesmus* sp. F51 displayed a lipid content of 22% during cultivation under optimal light stress at 938 μmol m^{-2} s^{-1}, while its lipid content was lower than 15% during cultivation at 700 μmol m^{-2} s^{-1} (Shen et al., 2018). Therefore, at moderate light intensity the Nordic *Desmodesmus* strains were capable of producing higher amounts of lipids compared to the thermotolerant *Desmodesmus* sp. F51 strain (Shen et al., 2018).

Salt stress significantly influenced the lipid content in both *Desmodesmus* strains. The maximal lipid content of 32.5% and 43.3% was achieved in *Desmodesmus* sp. (2–6) and (RUC-2), respectively, during moderate salt stress (8 g L^{-1} of NaCl) (Fig. 3b). In comparison to the control, the lipid content at these conditions increased by 2.1- and 1.6-fold in *Desmodesmus* sp. (2–6) and (RUC-2), respectively. A further increase in the NaCl concentration to 1.6 g L^{-1} decreased the lipid content by 30% and 25%, for *Desmodesmus* sp. (2–6) and (RUC-2), respectively. Excess Na^+ ions can reduce the electron exchange rate and damage the photosynthetic apparatus, which might lead to detachment of the PsbP protein of Photosystem-II (Srivastava et al., 2017). Therefore, Na^+ ions have a significant role in osmoregulation and metabolic processes in algal cells. Moreover, at its optimal concentration (8 g L^{-1}) of NaCl *Desmodesmus* sp. (RUC-2) was able to produce 1.3 times more lipids compared to *Desmodesmus* sp. (2–6). Srivastava et al. (2017) reported that the lipid content in *Chlorella sorokiniana* CG12 and *Desmodesmus* GS12 was increased by 1.5 to 2-fold at optimal salt stress. The lipid content was increased up to 33% in *Acutodesmus dimorphus* during salt stress of ~ 11.7 g L^{-1} NaCl, an 1.5-fold enhancement in comparison to growth in the absence of salt (Chokshi et al., 2017). Similarly, Ji et al. (2018) reported that *Scenedesmus obliquus* XJ002 was able to accumulate up to 32% lipids during salt stress of ~ 11.7 g L^{-1} NaCl, which was 2.5-fold higher than the control. Hence, moderate salinity can stimulate the lipid production in microalgae, however, the salt concentration of moderate stress varies between species. Too high salinity on the other hand reduces the lipid content in microalgae.

3.3. The effect of light and salt stress on the fatty acid profile

The fatty acid (FA) composition in the biomass of both Nordic *Desmodesmus* sp. strains cultivated under abiotic stress was determined after 14 days of growth (Fig. 4). As the FA profiles of the two Nordic *Desmodesmus* sp. had not been previously reported, gas chromatography-mass spectrometry (GC–MS) was used for FA analysis. GC–MS provides structural information and has well-established databases for FA identification with higher efficiency and selectivity in comparison to the simpler gas chromatography with flame ionization detection (GC-FID). Palmetic acid (C16:0), oleic acid (C18:1) and linolenic acid (C18:3) were the dominating FAs in both *Desmodesmus* sp. strains; a similar observation had been reported for biomass from *Desmodesmus abundans* (Xia et al., 2014). Increasing light stress from 100 to 1200 μmol m^{-2} s^{-1} slightly induced the synthesis of C16:0 in *Desmodesmus* sp. (2–6), while the content of C16:0 decreased in *Desmodesmus* sp. (RUC-2) under the same growth conditions (Fig. 4a). Alternatively, the content of C18:1 increased in *Desmodesmus* sp. (RUC-2) at higher light intensities, while the C18:1 content in *Desmodesmus* sp. (2–6) diminished by 75% during growth in very high light stress (1200 μmol m^{-2} s^{-1}). Nzayisenga et al. (2020) observed that increasing light from 50 to 300 μmol m^{-2} s^{-1} enhanced the C18:1 content in *Desmodesmus* sp. Stronger light intensity significantly increased the content of C18:3 and stearidonic acid (C18:4) in the biomass of *Desmodesmus* sp. (2–6), while the content of these FAs was diminished in biomass of *Desmodesmus* sp. (RUC-2) in the same growth conditions (Fig. 4a). These FAs belong to the group of ω-fatty acids, which due to their health benefits are considered as high value (Prasad et al., 2021). Light stress therefore promotes the synthesis of high value compounds in *Desmodesmus* sp. (2–6).

The presence of different salt concentrations during growth at 400 μmol m^{-2} s^{-1} affected the FA profile of the two strains. High salinity stress (16 g L^{-1} NaCl) enhanced the C16:0 content by 15% and 22% in *Desmodesmus* sp. (2–6) and (RUC-2), respectively, while moderate salinity stress did not induce any significant differences (Fig. 4b). The maximal content of C16:0 was 30% and 37% in *Desmodesmus* sp. (2–6) and (RUC-2), respectively, highest among all FAs. In both *Desmodesmus* sp. strains the content of C18:3 slightly improved with moderate salinity stress (up to 8 g L^{-1} NaCl), but diminished at higher salt concentrations (16 g L^{-1} NaCl). Singh et al. (2020) observed that the C18:3 content was elevated up to 13% in *Desmodesmus* sp. JS07 with increasing concentrations of different cytokinins. Therefore, different abiotic stresses can stimulate the synthesis of specific FAs in *Desmodesmus* sp.

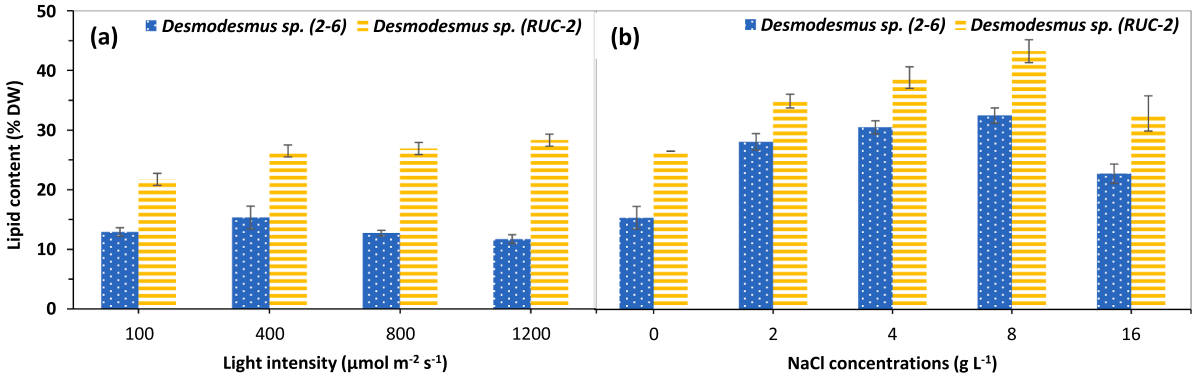

Fig. 3. Lipid content in Nordic microalgae *Desmodesmus* sp. (2–6) and (RUC-2) biomass after 14 days of growth in (a) light stress and (b) salt stress at 400 μmol m^{-2} s^{-1}.

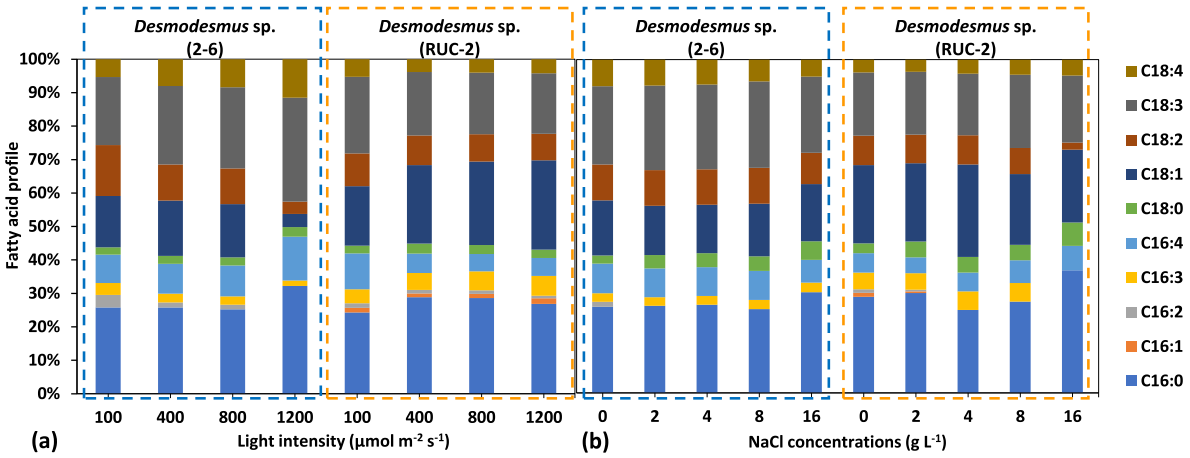

Fig. 4. FA profile of Nordic microalgae *Desmodesmus* sp. (2–6) and (RUC-2) biomass under abiotic stress (a) light stress and (b) salt stress at 400 µmol m^{-2} s^{-1}.

3.4. FTIR analysis of microalgal biomass after exposure to light and salt stress

FTIR analyses were performed in parallel to the pigment and lipid analysis to assess the overall effect of light and salinity stress on microalgal biomass composition (see supplementary material). In accordance with the lipid analysis the biomass of *Desmodesmus* sp. (2–6) did not show significant changes in the fingerprint region for lipids or fatty acids when exposed to different light intensities. Biomass harvested after very high light stress treatment (1200 µmol m^{-2} s^{-1}) showed a slight increase at 1740 cm^{-1}, which represents the C = O stretching vibrations of esters or fatty acids (Duygu et al., 2012). Nonetheless, this observation did not allow correlations to any special molecule in the biomass. Also the 2nd derivative spectra did not reveal major changes in the lipid- or carbohydrate-related areas, except a slight increase in carbohydrates in *Desmodesmus* sp. (2–6) biomass when exposed to 800 µmol m^{-2} s^{-1}. Interestingly, light stress seemed to have a larger impact on the biomass composition of *Desmodesmus* sp. (RUC-2) than on *Desmodesmus* sp. (2–6). FTIR data confirmed that increasing light (800 µmol m^{-2} s^{-1} and 1200 µmol m^{-2} s^{-1}) leads to an increase of carbohydrates and lipids in *Desmodesmus* sp. (RUC-2). Similarly, Nzayisenga et al. (2020) reported that increasing light intensity enhanced the carbohydrate fraction in *Desmodesmus* sp.

When exposed to increasing salt concentrations the lipid content increased in the biomass of both strains compared to the control. All spectra of biomass exposed to salinity stress displayed changes in the C = O stretching vibrations as well as in the lipid bending vibrations of methyl-groups in saturated and unsaturated fatty acids. These observations confirm the results on lipid content and fatty acid profiling of both *Desmodesmus* sp. strains (Figs. 3 and 4). The spectra of *Desmodesmus* sp. (RUC-2) biomass confirm lipid accumulation during salinity stress at 2, 4 and 8 g L^{-1} of NaCl. The peak correlated to the C = O stretching vibrations of fatty acids increased at higher salt concentrations along with the peaks correlated to lipid bending vibrations of saturated and unsaturated fatty acids (see supplementary material).

3.5. Effect of light and salt stress on pigments and carotenoids

Carotenoids and chlorophylls were identified in the freeze-dried biomass of both Nordic *Desmodesmus* sp. (2–6) and (RUC-2) strains after exposure to light and/or salt stress. In *Desmodesmus* sp. (2–6) increased light intensities led to the accumulation of total carotenoids, while in *Desmodesmus* sp. (RUC-2) the highest concentration of carotenoids was detected at a light intensity of 100 µmol m^{-2} s^{-1}. To investigate the presence of specific carotenoids, biomasses from *Desmodesmus* sp. (2–6) and (RUC-2) grown at their individual optimal growth conditions for biomass production were analysed by HPLC (Table 1). The maximum amount of total carotenoids was produced by *Desmodesmus* sp. (RUC-2) and *Desmodesmus* sp. (2–6) at moderate light stress of 400 µmol m^{-2} s^{-1} (~ 8.92 mg g_{DW}^{-1} or ~ 44 mg L^{-1} and ~ 4.64 mg g_{DW}^{-1} or ~ 20 mg L^{-1}, respectively). Growth at moderate light stress therefore is a promising approach to induce carotenoid production. *Desmodesmus* sp. was shown to increase its biomass productivity and lutein content at light stress up to 300 µmol m^{-2} s^{-1} (Xie et al., 2013). The heat-tolerant *Desmodesmus* sp. F51 even was able to produce high lutein content at mild light stress of 150 µmol m^{-2} s^{-1} (Xie et al., 2013), similar to *Desmodesmus* sp. (RUC-2) in this study. *Desmodesmus* sp. (RUC-2) therefore is able to produce slightly higher amounts of carotenoids compared to other *Desmodesmus* strains (Xie et al., 2013).

The total amount of carotenoids was also studied in the biomass of the two strains exposed to salt stress (at moderate light stress of 400 µmol m^{-2} s^{-1}). *Desmodesmus* sp. (2–6) produced a higher amount of total carotenoids (~ 12.5 mg g_{DW}^{-1} and ~ 41 mg L^{-1}) under combined light and salt (4 g L^{-1} NaCl) stress (Table 1), the total carotenoid content was 2.7-fold higher compared to the control grown at moderate light stress (400 µmol m^{-2} s^{-1}) in the absence of salt. *Scenedesmus* species contained an increased total carotenoid amount when cultivated under salt stress, the optimal concentration being 1% of NaCl (Elloumi et al., 2020). Although these salt concentrations are higher than the ones used in this study, the Nordic *Desmodesmus* sp. (RUC-2) and specially *Desmodesmus* sp. (2–6) produced higher amounts of total carotenoids compared to these *Scenedesmus* species. Some microalgae are known to use other osmoprotectants, such as saturated lipids or glutathione, to protect themselves against salt stress (Singh et al., 2018). Salinity stress in microalgae can change the membrane permeability or damage the membrane, which influences the integrity, fluidity and ion transport selectivity. Therefore, high salinity stress consequently leads to declined biomass production as well as synthesis of intracellular biomolecules.

As shown in Table 1, lutein was the dominating pigment in both *Desmodesmus* sp. strains followed by *cis*-neoxanthin/loroxanthin, violaxathin and zeaxanthin. The content of β-carotene was relatively low in both *Desmodesmus* sp. strains compared to other green algae, such as *Dunalliella salina* or *Chromochloris zofingiensis* (Varela et al., 2015). The maximal lutein content was 8.09 mg g^{-1} in the biomass of *Desmodesmus* sp. (2–6) and 5.97 mg g^{-1} in the biomass of *Desmodesmus* sp. (RUC-2) during cultivation in light and salt stress. In both *Desmodesmus* sp. strains salinity stress enhanced the lutein content. *Desmodesmus* sp. (RUC-2) produced higher amounts of β-carotene compared to *Desmodesmus* sp. (2–6), while *Desmodesmus* sp. (2–6) instead biosynthetized higher amounts of zeaxanthin, which increased with increasing light intensities. Combined optimal stress of light and salinity enhanced the zeaxanthin content by 3- and 3.5-fold in *Desmodesmus* sp. (2–6) and (RUC-2), respectively compared to the control (moderate intensity (400 µmol m^{-2} s^{-1}) in the absence of salt). *Scenedesmus* sp. SVMIICT1 was

Table 1
Relative quantities of carotenoids and chlorophylls in *Desmodesmus* strains (2–6) and (RUC-2) grown under their optimal conditions.

Optimal growth conditions		Pigments content (mg g^{-1})								Total Carotenoid production (mg L^{-1})
		Chlorophylls		Cis-neoxanthin + Loroxanthin	Violaxanthin	Anthera-xanthin	Lutein	Zeaxanthin	β-Carotene	Total Carotenoids
		a	*b*							
Desmodesmus sp. (2–6)	400 µmol m^{-2} s^{-1}	6.66 ± 0.45	7.02 ± 0.82	0.30 ± 0.01	0.46 ± 0.05	0.07 ± 0.03	3.13 ± 0.53	0.56 ± 0.07	0.13 ± 0.01	4.64 ± 0.60	16.61 ± 0.6
	1200 µmol m^{-2} s^{-1}	6.79 ± 0.84	9.50 ± 0.21	0.41 ± 0.03	0.55 ± 0.12	0.13 ± 0.01	3.54 ± 0.36	0.73 ± 0.14	0.19 ± 0.02	5.54 ± 0.68	19.94 ± 0.68
	400 µmol m^{-2} s^{-1} + 4 g L^{-1} NaCl	10.8 ± 0.74	11.6 ± 1.49	0.68 ± 0.05	0.96 ± 0.12	0.65 ± 0.20	8.09 ± 0.96	1.72 ± 0.66	0.42 ± 0.07	12.52 ± 2.01	40.93 ± 2.01
	100 µmol m^{-2} s^{-1}	15.0 ± 0.39	14.3 ± 1.98	2.13 ± 0.13	1.29 ± 0.06	0.07 ± 0.03	5.58 ± 0.77	–	1.09 ± 0.15	10.16 ± 0.51	32.29 ± 0.51
Desmodesmus sp. (RUC-2)	400 µmol m^{-2} s^{-1}	10.5 ± 1.16	11.0 ± 0.79	0.85 ± 0.07	0.94 ± 0.10	0.10 ± 0.02	5.74 ± 0.19	0.28 ± 0.14	1.01 ± 0.01	8.92 ± 0.19	43.81 ± 0.19
	400 µmol m^{-2} s^{-1} + 8 g L^{-1} NaCl	4.56 ± 0.11	12.1 ± 0.09	0.89 ± 0.19	0.54 ± 0.03	0.06 ± 0.01	5.97 ± 0.39	0.99 ± 0.09	0.32 ± 0.02	8.78 ± 0.49	37.67 ± 0.49

reported to produce a relatively high amount of lutein (1.43 mg g^{-1}) at a light intensity of 50 µmol m^{-2} s^{-1} (Kona et al., 2021). Impressively, in this study both *Desmodesmus* sp. (2–6) and (RUC-2) were able to produce 5.6- and 3.9-fold higher lutein content, respectively, compared to *Scenedesmus* sp. SVMIICT1. Therefore, it is worth exploring Nordic *Desmodesmus* sp. strains further for pilot-scale cultivation to produce high value compounds used in various industrial applications. Seawater with moderate salt concentrations (e.g. Baltic seawater) will provide an excellent and cheap cultivation medium.

4. Conclusion

Abiotic stress stimulated the production of biomass and high value compounds in the two Nordic *Desmodesmus* strains (2–6) and (RUC-2). A combination of light and salinity stress improved the lipid and carotenoid content in *Desmodesmus* sp. (2–6) (2.1- and 2.7-fold, respectively). Also, *Desmodesmus* sp. (RUC-2) produced most lipids (43.2%) when exposed to both stresses, however, maximal carotenoid production (~ 44 mg L^{-1}) was achieved at moderate light stress (400 µmol m^{-2} s^{-1}) in the absence of salt. Cultivation of both Nordic microalgal strains in Baltic seawater will open the possibility to produce high value products for food and feed.

CRediT authorship contribution statement

Sanjeet Mehariya: Conceptualization, Methodology, Investigation, Validation, Data curation, Formal analysis, Visualization, Writing – original draft. **Martin Plöhn:** Conceptualization, Data curation. **Antonio Leon-Vaz:** Data curation, Validation. **Alok Patel:** Data curation, Visualization, Validation. **Christiane Funk:** Funding acquisition, Project administration, Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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