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Neocystis mucosa, a polar alga producing oils containing polyunsaturated essential fatty acids

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ABSTRACT

The sustainable cultivation of microalgae for bioactive compounds, such as polyunsaturated fatty acids (PUFAs), is vital for addressing global health and nutritional challenges. However, the availability of algal strains suitable for year-round cultivation in temperate climates is limited. This study investigates the psychrotolerant green alga *Neocystis mucosa* (strain Šnokhousová and Elster, CCALA No. 1141), isolated from Svalbard soil, as a promising candidate for outdoor cultivation in Mid-European climates.

Molecular analysis confirmed the identification of the strain as *N. mucosa*. It demonstrated optimal growth at 12 °C under low light intensity (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$). However, it tolerates short-term light and temperature fluctuations (5 °C to 25 °C). This resilience ensures that there is no significant risk of cell death due to temperature and light variations, such as those that occur during sunny winter days. Nighttime LED lighting increased growth by 160 % compared to sunlight alone, yielding biomass production rates of 1.73 $g \cdot m^{-2} \cdot d^{-1}$ and 0.135 $g \cdot L^{-1} \cdot d^{-1}$, with a maximum dry matter of 6.3 $g \cdot L^{-1}$. The strain displayed high lipid productivity, with unsaturated fatty acids comprising 51–63 % of total fatty acid content. Notable components included linoleic acid (14.9–17.1 %), α-linolenic acid (18.3–23.1 %), and hexadecatetraenoic acid (10.2–16.7 %). Lipid and PUFA percentages increased with culture age.

This study highlights the practical potential of polar *N. mucosa* for year-round cultivation in temperate climates, offering a sustainable source of PUFAs for food supplementation and immunomodulation, including reducing risks associated with COVID-19. These findings advance biotechnological approaches to sustainable PUFA production, addressing global dietary needs.

1. Introduction

In recent years, the cultivation of microalgal biomass has gained significant attention due to its diverse applications, ranging from the production of alternative foods and nutritional supplements to its use in energy production and wastewater treatment. Microalgae are particularly valued in the context of a circular economy, offering sustainable solutions to meet global demands [1]. Among the many bioactive compounds derived from microalgae, polyunsaturated fatty acids (PUFAs), including essential fatty acids (EFAs) such as α-linolenic acid (ALA) and linoleic acid (LA), are of particular importance for human health. These fatty acids play a vital role in numerous biological processes, including the biosynthesis of prostaglandins and other signaling molecules, yet humans and most mammals are unable to synthesize them efficiently [2,3].

Despite their critical role, the dietary imbalance of ω-6 and ω-3

Abbreviations: DM, dry matter; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EFA, essential fatty acids; FA, fatty acids; GLA, γ-linolenic acid; HDA, hexadecatrienoic acids; HDTA, hexadecatetraeneoic acid; LA, linolenic acid; OL, oleic acid; PA, palmitic acid; PAR, photosynthetically active radiation; PUFA, polyunsaturated fatty acids; SDA, stearidonic acid; SFA, saturated fatty acids. Trivial and systematic names see also Table 2 Supplement..

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PUFAs caused by the widespread consumption of vegetable oils has led to adverse health outcomes, such as increased risks of cardiovascular diseases, cancer, and autoimmune disorders [4,5]. This growing concern highlights the need for alternative sources of ω-3 PUFAs to restore the balance and mitigate these risks. Recent studies have also pointed to the potential role of ω-3 PUFAs, such as DHA and EPA, in managing inflammation and reducing risks associated with diseases like COVID-19 [6,7].

Microalgae present a promising solution as natural, sustainable sources of PUFAs [8–10]. While species like *Chlorella* and *Desmodesmus* are commonly cultured, their ω-6 to ω-3 ratios and growth characteristics limit their suitability for large-scale, year-round cultivation in temperate regions [11]. Additionally, the cultivation of microalgae in open systems poses challenges such as temperature fluctuations, light availability, and contamination, particularly during colder months when growth is inhibited [12].

To address these limitations, there is a pressing need to identify robust microalgal species capable of thriving under temperate-zone conditions, including during winter, while producing high-value compounds like PUFAs. Existing studies have largely focused on cultivation either under sunlight or artificial light, with limited exploration of integrated approaches that extend the growth period [13,14]. Moreover, pilot-scale experiments remain scarce, particularly for novel species with advantageous traits.

Microalgae bioprospecting, particularly in low-temperature environments, plays a crucial role in advancing algae biotechnology and fostering sustainable innovation. Polar microalgae developed a range of ecological, physiological, and molecular strategies to adapt to the extreme conditions of polar environments [15]. These adaptations involve the production of a wide variety of compounds derived from different metabolic pathways, providing protection against stress induced by the harsh conditions [16]. It garnered significant biotechnological interest across various industries and enables the development of new technologies that support local communities and promote the growth of locally led emerging businesses in Arctic regions [17].

In this study, we explore the potential of *Neocystis mucosa*, a microalga with promising traits, for pilot-scale cultivation. This research represents a novel effort to assess its growth and PUFA production capabilities in outdoor systems under temperate climatic conditions. By demonstrating its resilience to low temperatures and its ability to grow autotrophically while resisting contamination, this study aims to establish *Neocystis mucosa* as a viable candidate for sustainable, year-round PUFA production. Furthermore, we investigate innovative cultivation strategies, including artificial lighting, to overcome seasonal limitations and improve productivity. This work contributes to advancing biotechnological solutions for addressing global dietary and health challenges through sustainable microalgal cultivation.

2. Methods

The investigated strain of *Neocystis mucosa* was isolated in Svalbard, from moraine soil, close to the glacier front, Andreasbreen, Spitsbergen west coast (78°36′25.45″N, 12°13′26.24″E, 277 m a.s.l., the sample was collected August 8th 2018), by standardized method described by Andersen [18] (Fig. 6 Supplement). The strain is deposited in the Culture Collection of Autotrophic Organisms of the Institute of Botany of the Czech Acad. Sci. Třeboň, under No. CCALA 1141, strain Šnokhousová and Elster 2024.

2.1. Pre-culturing

The strain was pre-cultivated under laboratory conditions from a slant culture in test tubes to 2 L bottles containing ½ SŠ liquid mineral medium [19], aerated with filtered air (Millipore, Midisart 2000, PT FE IN, pore size 0,2 μm) containing 2 % CO_2 and irradiated using a white LED panel (Optonica LED panel light DL2358, Bulgaria) with a light intensity of 180 μmol.m^{-2}.sec^{-1} (only a half of this for the first few days) and at room temperature.

2.2. Light microscopy

The alga was photographed under an Olympus BX 51 microscope, equipped with a 100× objective, HI, 1.35, and digital camera DP74. The microphotographs were processed using Olympus cellSens Entry 3.1 software (Olympus, Japan).

2.3. Molecular taxonomy

DNA extraction, PCR and sequencing, secondary structure prediction of nuclear ITS2.

Total genomic DNA was extracted according to Procházková et al. [20]. The 18S small subunit ribosomal RNA gene (18S rRNA), nuclear rDNA internal transcribed spacer region 1 (ITS1), 5.8S rRNA gene and nuclear rDNA internal transcribed spacer 2 (ITS2) were amplified from DNA isolates by polymerase chain reaction using existing primers 18F2 (AACCTGGTTGATCCTGCCAGT) and 18R2 (TGATCCTTCTGCAGGTTCACCTACG) [21], SSU (CTGCGGAAGGATCATTGATTC) and LSU (AGTTCAGCGGGTGGTCTTG) [22]. The amplification and sequencing reactions for these markers were identical to those described by Procházková et al. [20]. The sequences obtained for CCALA 1141 were submitted to the GenBank genetic sequence database at the National Centre for Biotechnology Information (NCBI) (ITS1 partial+5.8S + ITS2 + 28S RNA gene partial: PP510219; 18S rRNA gene: PP506672). The annotation and prediction methods for the secondary structure of the nuclear ITS2 region were the same as those previously described by Remias et al. [23].

2.4. Crossed gradients of temperature and light

The method of cultivation in crossed temperature and light gradients [24–27] was performed by exposure of glass Petri dishes containing liquid medium (15 mL for each dish). The light was placed obliquely above the third side perpendicularly, and thus all combinations of light and temperature were created. The dishes were covered with their lids and CO_2 was added to a concentration of approximately 1–2 %. At the end of cultivation, the suspension was centrifuged, frozen, lyophilized and weighed. Irradiation (PAR; photosynthetically active radiation, i.e. 420–700 nm wavelengths) was evaluated using a LiCor LI-250A light meter equipped with a LI-190 quantum sensor (both LiCor, USA) and temperature was measured by a Testo 110 digital thermometer (Testo SE & Co., Germany). The temperature gradient ranged from 12 to 23 °C, and the irradiance gradient from 36 to 258 μmol m^{-2} s^{-1}.

2.5. Pilot-plant cultivation

After growth, the inoculum was transferred to a cultivation unit (BSC Engineering Brno, CZ), after Doucha et Lívanský [28], volume 150 L, area 12 m^2, length 12 m, slope 15 cm, suspension thickness 15 mm, and placed in a greenhouse where the temperature was controlled above 8 °C (Fig. 1 Supplement). The liquid mineral medium [19] was diluted by ½ with tap water, and CO_2 was administered by pumping at about 5 L. min^{-1}. Temperature and irradiation were recorded continuously at 10 min intervals by Minikin QTie (air temperature and PAR) and Tie (suspension temperature) dataloggers (EMS, Brno). The growth curve was evaluated every day by measuring the absorbance at 750 nm using a Shimadzu UV-1650 PC spectrophotometer in 1 cm cuvettes. Biomass was harvested by centrifugation using an EVODOS 10 centrifuge at 7000 rpm, frozen at −20 °C and lyophilized at 0.05 hPa. Dry matter (DM) was evaluated during cultivation by centrifugation for 20 min at 3000 ×*g*, in pre-weighed 2 mL Eppendorf tubes and drying to the constant weight at 105 °C.

The alga was cultivated in a pilot plant unit (Fig. 1 Supplement) as

described above, in the periods of 23.4. - 14.5.2021, 16.5. - 28.6.2021, 7.1.-14.4.2022 and 14.4.-1.6.2022 (three repetitions). For the suspension temperature and PAR oscillations see Figs. 2–5, Table 1, all in Supplement.

To support sterile conditions during cultivation, the pilot plant unit was sterilized prior to cultivation with commercially available chlorine algaecide (1 L/150 L of water). Further, the pilot plant unit was closed in a greenhouse. Importantly, the starting inoculum was dense enough to assure the viability and robustness of the cultivated strain. Also, the selected *N. mucosa* has a high growth rate and a low minimal growth temperature which makes contamination by other species difficult.

The cost of dry matter production in electricity was calculated as gravimetrically determined dry matter multiplied with the content of the platform and divided by the electricity consumption for the date (Table 1).

2.6. LED sources

Type THOME (Optimal LED 134 W DALI), with white, daylight, thin longitudinal panels provided little shade and allowed the platform to be illuminated by the Sun during the day, and small lenses in front of point sources focused the light exactly on the surface of the unit. The LEDs were automatically switched on or off by a twilight switch (Cd-DALI / DSI, BEG Brück Electronic GmbH) at the threshold sun intensity of 20 $\mu mol.m^{-2}.s^{-1}$. The intensity of the LED lights measured in the cultivation area was 1000 $\mu mol.m^{-2}.s^{-1}$. The power consumption of one panel was 98 W.

2.7. Identification of fatty acids

The samples for analysis of fatty acids were taken during the stationary phase of cultivation in the pilot plants. Freeze-dried biomass of 10 mg weight was soaped with a 10 % solution of KOH in methanol at room temperature overnight. Neutral compounds were extracted from a solution at pH 9 by shaking with diethyl ether. The aqueous solution containing fatty acids was acidified to pH 2 and the acids were subsequently extracted into hexane. Fatty acids were methylated in a mixture of BF_3/methanol and identified using GC–MS, i.e., by gas chromatography - mass spectrometry. The sample was injected into a capillary column (Supelcowax 10; 60 m × 0.25 mm ID, 0.25 mm film thickness; Supelco, Prague) and for elution, the temperature was maintained at 50 °C for 5 min, then a gradient was initiated by heating the column at a rate of 10 °C min^{-1} to 240 °C and then holding isothermally for 15 min at 240 °C. The carrier gas was helium at a flow rate of 0.52 $mL.min^{-1}$. All spectra were scanned in the range of 50 to 600 Da. The structure of methyl esters was determined on the basis of retention times, their fragmentation [29] and by comparison of the mass spectra with the mass spectra of commercial standards obtained from Merck (formerly Sigma Aldrich, Prague, Czech Republic).

2.8. Statistics

The microclimate descriptive statistics calculation and comparison between the pilot plants were performed using Statistica 14.0 software (TIBCO Software, USA). The outliers selection and curve fittings were performed using Matlab SW 2021a (MathWorks, USA) and Sigmaplot 14.0 softwares (Systat, USA). The generalized additive models were fitted using CANOCO 5.0 [30]. The results were considered statistically significant for $P < 0.05$.

3. Results and discussion

3.1. Identification of the strain

3.1.1. Morphology

Neocystis mucosa belongs to the group of green coccal algae (Chlorophyta, Chlorophyceae, Sphaeropleales) with bean-shaped to ovoid cells, dimensions 5 × 8 μm, containing a few parietal chloroplasts without a pyrenoid. This was confirmed by light microscopy. They reproduce asexually, by longitudinal division into several autospores, most often 2–4 (Fig. 1) and the morphology of the cells was consistent with the description of the species [31]. Under LED irradiation the cells were smaller and faded.

3.1.2. Molecular taxonomy

With the NCBI basic local alignment search tool server, the 18S rRNA gene sequence of strain CCALA 1141 was found to be identical to that of strain SAG 40.88 (JQ920367.1, 1610 out of 1610 bp, [32]) and ITS2 of strain CCALA 1141 differed by only one bp from SAG 40.88 (Fig. 2). This closely related strain SAG 40.88 was isolated by Broady cca in 1973 from unknown material from Signy Island, Antarctica, initially assigned to *Ellipsoidon* sp., but later transferred to *Nephrodiella minor* Pascher and then recently assigned to *Neocystis mucosa* Krienitz, Bock, Nozaki & Wolf (Chlorophyta, Trebouxiophyceae) [32]. Strain CCALA 1141 is also closely related to strain CCAP 204/1 (authentic strain for *Neocystis mucosa*) from an unknown habitat in Germany (2 bp difference out of 184 bp ITS2, MK541791), CAUP D 801 strain from Antarctica soil, probably from the McMurdo Sound region (3 bp difference, JQ920366), strain KR 1989/14 from unknown material in Germany, Lake Feldberger Haussee (4 bp difference, JQ920365), S2CENT-38 snow airborne strains from southern Sweden (10 bp difference, MK005068.1) [33], and K2 and K1 strains isolated from volcanic soils in Kamchatka Peninsula (6 bp difference, OM522658) [34,35]. As a consequence, the studied alga CCALA 1141 was assigned to *Neocystis mucosa* (Fig. 1).

3.2. Optimal growth conditions

The growth of *Neocystis mucosa* in crossed gradients of temperature and light was driven mainly by temperature (Interactive Forward Selection analysis explaining 66.4 % of the observed variation, pseudo-F = 55.3, $P = 0.002$). Since the alga was isolated from a cold location, Svalbard, the strain preferred low temperatures below 15 °C, like other polar strains of *Phormidium*, *Pinnularia*, and *Zygnema* [36], *Chlorella*-like green coccal algae [37] and *Stichococcus* [38]. Growth was suppressed at higher temperatures, but the strain was still able to grow, even at 24 °C (Fig. 3; GAM model F = 38.5, $p < 0.00001$). After Hoham [39] and Morita [40], this cannot be described as a true psychrophilic alga, but rather as a psychro-tolerant one, making it prospective for algal biotechnology in the Polar Regions [41].

The optimal irradiance was relatively low, ca 100 $\mu mol.m^{-2}.s^{-1}$, corresponding to the strain origin from soil, however these effects were not statistically significant (Fig. 3; GAM model F = 0.7415, p =

Table 1
Comparison of the growth of the alga *Neocystis mucosa* on a platform illuminated only by the Sun and a one illuminated at night with LED lights, with an evaluation of electricity consumption. Dry-matter (DM) was determined gravimetrically. Yield of dry matter was calculated from DM max. in 150 L.

	From	To	Days	DM max	DM yield	DM/m^2/day	DM/L/day	kWh	kWh/day	kWh/g DM
Sun + LEDs	12.1.22	9.2.22	30	17.24	2586	7.18	0.575	1308.69	43.62	0.51
	11.2.22	12.4.22	37	11.64	1746	3.93	0.314	1572.3	42.49	0.9
	14.4.22	3.5.22	20	9.99	1499	6.25	0.499	645	32.25	0.43
Sun	10.1.22	28.3.22	50	6.89	1033	1.7	0.138	–	–	–

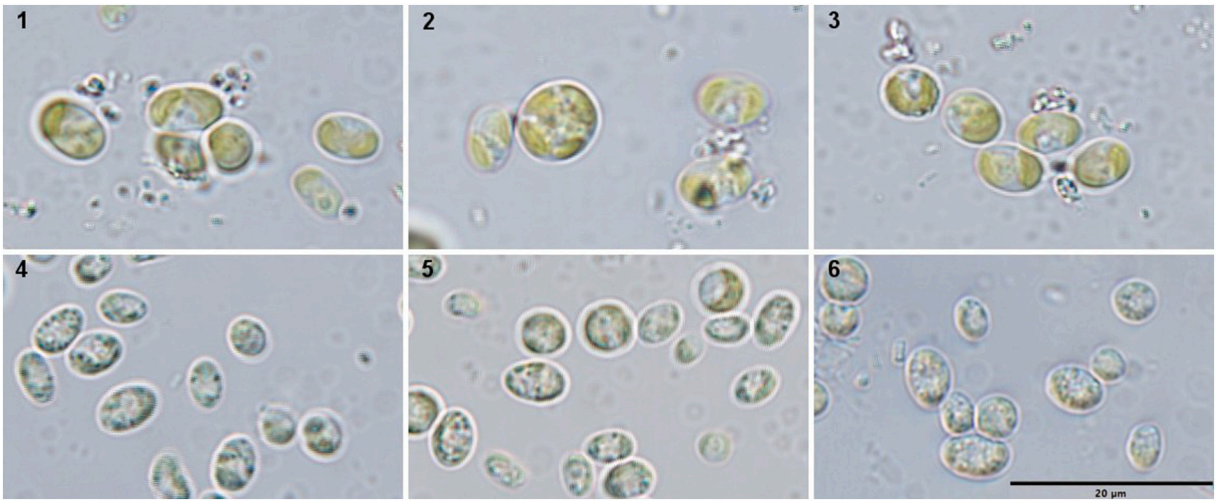

Fig. 1. The alga *Neocystis mucosa* under a light microscope; from pilot-plant units: The Sun only (1–3) and the Sun+LEDs (4–6), (22.I.2022). Bar is 20 µm.

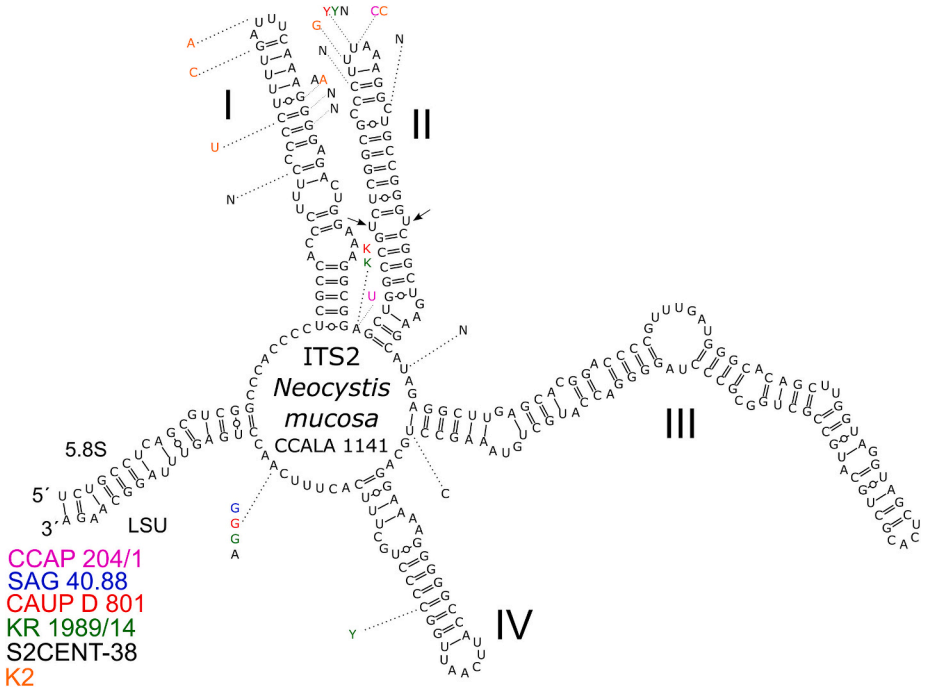

Fig. 2. Comparison of the secondary structure of ITS2 between strain CCALA 1141 (PP510219, this study) and *N. mucosa* type strain CCAP 204/1 (MK541791.1), the strains SAG 40.88 (JQ920367), CAUP D 801 (JQ920366), KR 1989/14 (JQ920365), S2CENT-38 (MK005068) and K2 (OM522658). Helices are labelled with Latin numbers. Nucleotide differences are outside the structure and linked by dotted lines. The relevant nucleotide differences are indicated in colors. No compensatory base changes between the CCALA 1141 strain and all the other strains, including the type strain, were found. Note the U-U mismatch in helix II (arrows).

0.51415). Similar preferences for low irradiances were also observed in other *Chlorella*-like polar soil algae [37]. Optimal conditions for maximum biomass and PUFA productivity of the polar microalga *Chlamydomonas malina* RCC2488 cultivated in photobioreactors was reported as 8 °C and 250 µmol.m^{-2}.s^{-1} [42]. The maximum growth and PUFA production in *Koliella antarctica* were reached at 15 °C and 250 µmol m^{-2} s^{-1} [43]. The general requirement for relatively low light corresponds to mean light conditions encountered during temperate winter or polar summer [44].

3.3. Pilot-plant cultivation

The pilot plant cultivation units used had a surface area of 12 m^2 and a volume of 150 L per unit (Fig. 1 Supplement). One of them had LED night irradiation. We cultivated the alga 3 times. The results, e.g. dry weight yields, and electricity consumption are summarized in Table 1. For the suspension temperature and PAR oscillations see Figs. 2–5, Table 1, all in Supplement.

The first period of growth, i.e. the exponential phase, is shown in Fig. 4, and while the complete growth curves are shown in Fig. 5. The exponential phase lasted 25 days for the unit irradiated only from the Sun and 20 days for the Sun +LEDs unit. The difference between the Sun and night irradiation from LEDs was marked. The complete growth curve is shown in Fig. 5 and is composed of two parts (fed-batch regime), followed by harvesting and spiking the rest with nutrients, without additional inoculation (semi-continuous regime). Only washing the unit with tap water and adding nutrients were sufficient to start a new growth phase. The possibility of fed-batch and semicontinuous

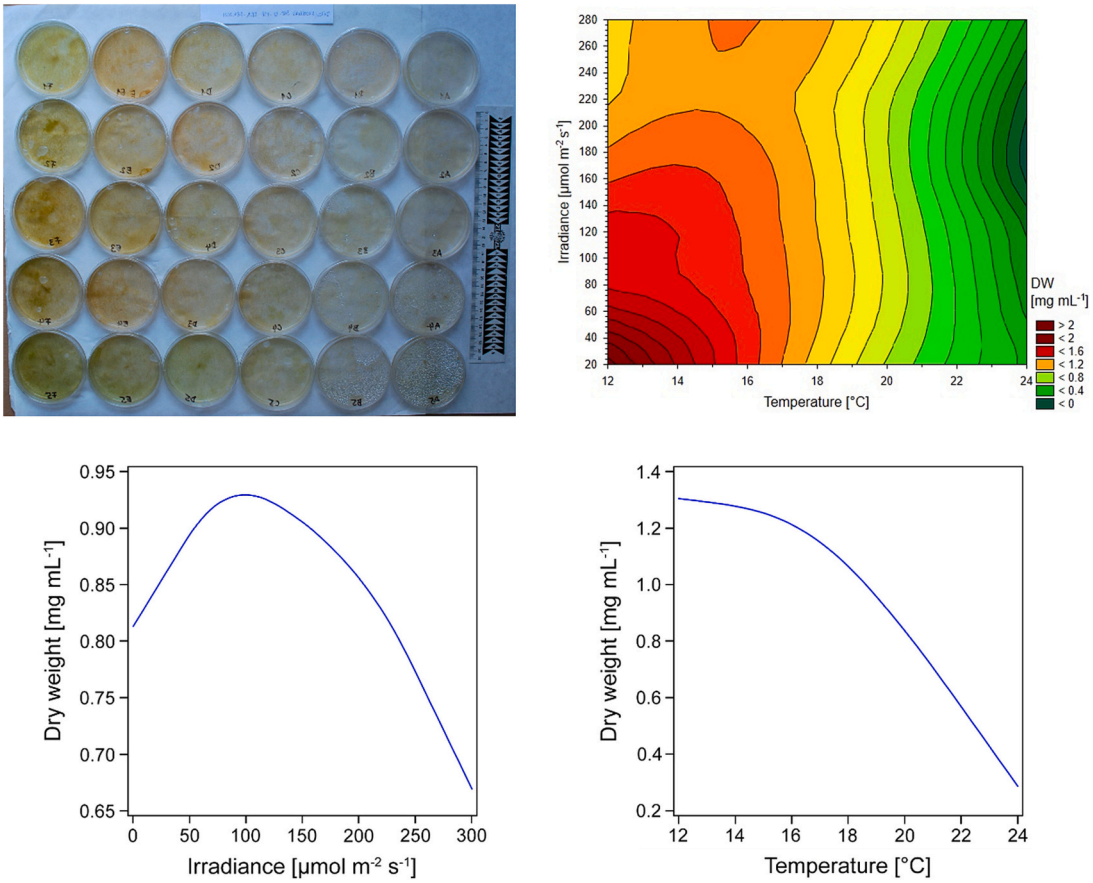

Fig. 3. Growth of the alga *Neocystis mucosa* in crossed gradients of temperature and light. (A) the photograph of Petri dishes with suspension (B) areal plot of the final biomass content (C) GAM model of dependency of dry weight content on temperature, and (D) GAM model of dependency of dry weight content on temperature.

cultivation was demonstrated.

By comparing pilot plant units illuminated only by the Sun and with night lighting (Fig. 4), we see an improvement in growth for the same cultivation time. In the linear phase of the growth curve, when growth was limited by light, fitting by linear regression resulted in equation for the variant illuminated at night $DM\left[g\,L^{-1}\right] = 0.927 \times t - 1.625$ ($R^2 = 0.996$) and for illuminated only by the Sun $DM\left[g\,L^{-1}\right] = 0.579 \times t - 1.025$ ($R^2 = 0.98$) where DM was the biomass content, t time and the growth rate corresponded to the slope lot the regression, i.e. 0.927 and 0.579 g L^{-1} d^{-1} for LED+Sun and Sun pilot plants, respectively (Fig. 4), so the incremental difference was 160 %. This was not as big as it was in the alga *Dictyosphaerium chlorelloides* [45], where the difference was even higher when it was irradiated with LEDs. For *N. mucosa* (CCALA 1141) the differences were only a few percent, in favor of the night illuminated platform with LEDs.

The *N. mucosa* (CCALA 1141) culture was harvested from Sun and LED units yielding a total of 975 g DM i.e. 1.73 g.m^{-2}.day^{-1} and with respect to volume, 0.135 g.L^{-1}.day^{-1}. In the Sun-illuminated platform, an increasing yield with time was achieved, 6.34–13.9 g.m^{-2}.day^{-1} and related to the volume of 0.51–1.11 g.L^{-1}.day^{-1}. The plateau lit only by the Sun had a significantly lower production of 1.52 g.m^{-2}.day^{-1} and 0.12 g.L^{-1}.day^{-1} (Tables 1, 2).

3.4. Production of lipids and PUFAs

The total lipid content was 14.2–19.5 % in dry matter on the platform illuminated only by the Sun and 17.4–24.4 % on the platform with additional night light (Table 2). The content of individual, biotechnologically interesting fatty acids, i.e. PA, OL, LIN, and ALA was around 20 % of total fatty acids (Table 3). The fatty acid compositions did not differ significantly for either platform, but production on the night lit platform was higher (Table 3). These values are comparable to similar snow and polar algae (Table 3), i.e. *Bracteacoccus bullatus* and *Chodatodesmus australis* [46,47]. The content of PUFAs was around 60 % of the total FAs in both cultivation modes, which is very promising for biotechnology. OL and ALA are precursors of EPA (the basic component in fish oil), which is valuable as a food and feed additive. PUFAs are also effective in reducing the risk of covid-19 [6,7]. To compare FA production with commercially available biomass of the marine alga *Chlorella vulgaris*, *N. mucosa* in this study had a lipid composition of approximately 23–34 % saturated fatty acids, 15–25 % monounsaturated fatty acids, and 42–62 % PUFAs as a percentage of total lipids (of which over 30 % are ω3 PUFAs) [48]. Commercially produced *Spirulina* contained 33.7–66.7 % SFA, 28.20–47.78 % PUFAs (ω 3 and ω 6 PUFA) and especially GLA, 4.07–22.51 % of the fatty acids. EPA and DHA were found at levels of only 1.79–7.70 %, and 2.28–2.88 %, respectively [49]. Both commercial microalgae had similar FA compositions as *N. mucosa* (CCALA 1141), however, *N. mucosa* was capable of growing at low temperatures during the annual cold season.

3.5. The cost of additional night lighting

The consumption of electrical energy, per gram of biomass, is presented in Fig. 6 and Table 1. It ranged from 0.5 to 1 kWh per 1 g DM. With a content of 50 % of PUFAs in dry matter, electricity consumption was 1–2 kWh per gram of PUFAs. This was comparable with literature data, where the green alga *Scenedesmus obliquus* produced 1 g of dry matter in the illuminated unit for approximately 0.2 kWh [14].

The cost of producing a gram of dry matter decreased exponentially with time. However, it is necessary to find the actual optimum for the

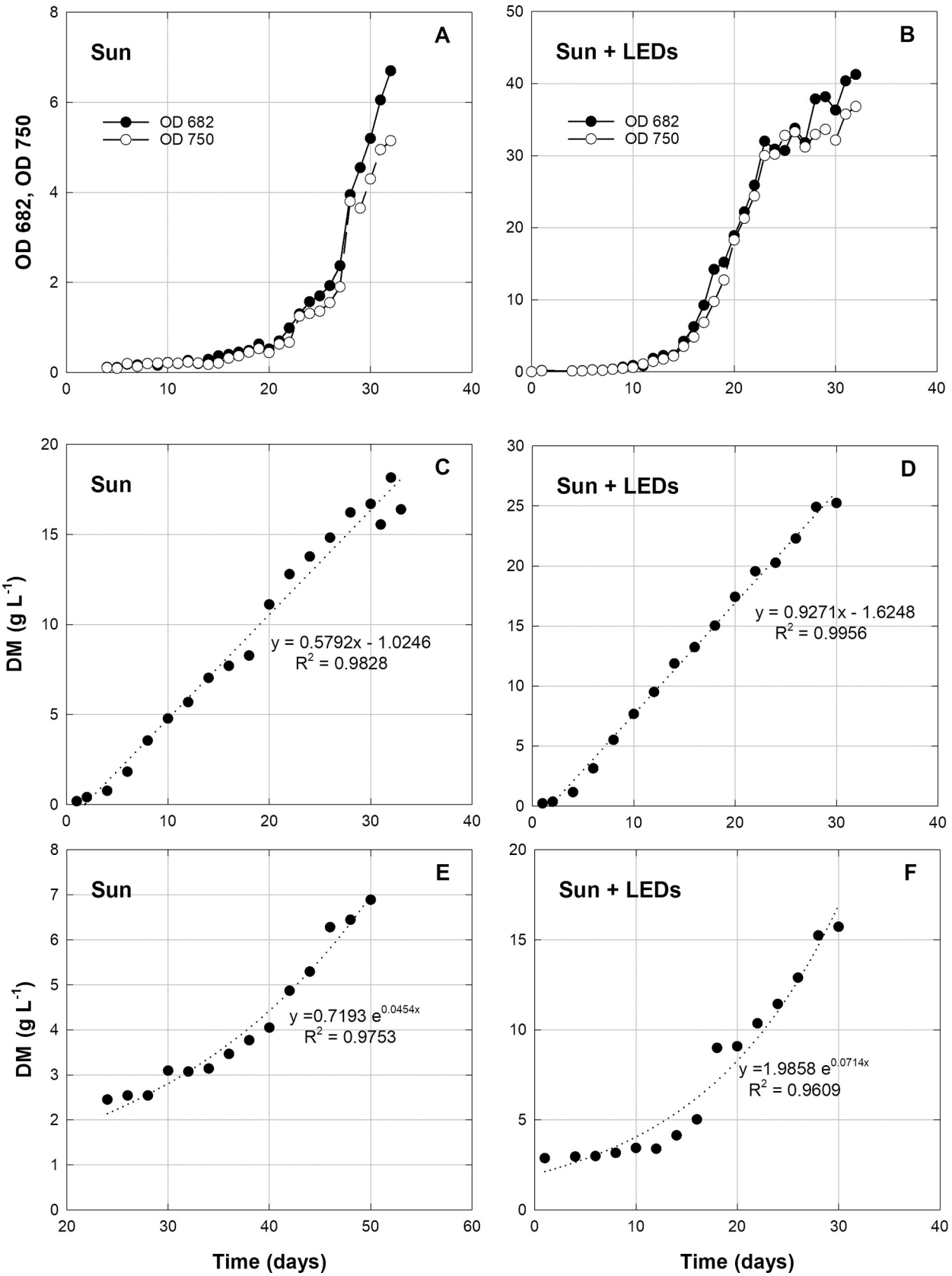

Fig. 4. Comparison of growth curves of the alga *Neocystis musosa* in pilot-plants, one irradiated only from the Sun (A, 6.1-30.2.2022) and irradiated by LEDs during night (B, 6.1.-14.2.2022) as OD 682 and 750 nm. Expressed also in dry matter, gravimetrically determined in linear parts (C, 16.5.-21.6.2021; D, 16.5.-15.6.2021). Comparison of exponential parts of the growth curves (E, 22.1.-3.2.2022; F, 16.5.-23.6.2021). Cultivation 16.5. – 28.6. (the Sun) and 16.5. – 23.6.2021 (S + LEDs).

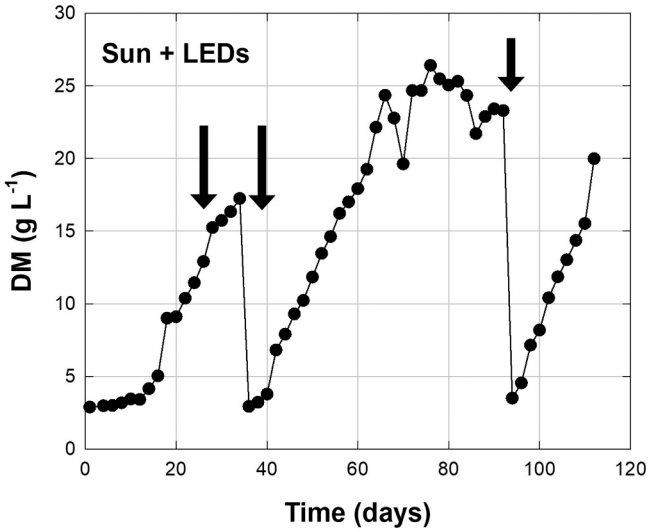

Fig. 5. Semi-continuous cultivation of the alga *Neocystis mucosa* on a pilot-plant unit, with night-time illumination. The suspension was harvested, supplemented with water and nutrients (fed-batch mode followed by semi-continuous mode). The remains of the suspension in the system were used as an inoculum. Dry matter was estimated gravimetrically. Arrows indicate nutrient additions and harvestings (feed-back mode) except for the first arrow where only nutrients were added (fed-batch modus). Cultivation 6.1. – 1.6.2022.

cost and profit curves. As the main problem was with microalgal PUFA production, except for potential safety risks of algal biomass and low ratios of ω-3/ω-6, the high cost of biomass production was defined [50]. The curve of electricity consumption dropped steeply until the 18th day of cultivation, then only gradually (Fig. 6). When electricity was used only during the night, some part of the energy was supplied from the Sun cost-free. *N. mucosa* (CCALA 1141) can be grown in fed-batch mode as well as in semi-continuous cultivation mode (Fig. 5). However, the harvesting of an older suspension is more economical due to a higher DM content.

4. Conclusions

In the realm of bioenergy research, the production of algal oil encounters a significant obstacle due to its high production costs. *N. mucosa*, the strain isolated from soil in Svalbard, has been shown to be suitable for cultivation on a pilot plant scale during the annual cold season. Its growth optima are at a low temperature (12 °C) and with low light (10 μmol.m^{-2}.s^{-1}). The high content of lipids and PUFAs (17.4–24.4 % in dry matter and approximately 60 % of total FA) is applicable for the production of food and feed additives. Night light with LEDs increased production by 160 % compared to cultivation units irradiated only by the Sun. The production costs in this case were comparable to those found in the literature and were approximately 0.5–1 kWh.g^{-1} of dry matter. Despite the daunting cost barrier currently faced by algal bioenergy, concentrating on the development and utilization of high-value products like PUFAs could render it more economically viable.

By employing the new polar *N. mucosa* strain in the pilot plant cultivation, this study showed the importance of low-temperature microalgae bioprospecting in advancing algae biotechnology and fostering sustainable innovation. By identifying and utilizing cold-adapted microalgae, cultivation periods in temperate climates can be extended during colder seasons enhancing sustainable algae biotechnology. Thus, the benefits extend globally, including for European societies and indigenous Arctic communities, by facilitating the innovative and sustainable food production, creating a more resilient and resource-efficient future.

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CRediT authorship contribution statement

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve English language and style. After using this tool/service, the author(s) reviewed and edited the content as needed and take (s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial

Table 2
Contents of total lipids, saturated, mono- and poly-unsaturated fatty acids of the alga *Neocystis mucosa* cultured on platforms illuminated only by the Sun and the Sun during the day and at night by LED sources. Individual FAs are listed in Table 3.

	5.5.21	10.5.21	24.5.21	3.6.21	10.6.21	22.6.21	Mean
Unit only by the Sun							
% lipids in DM	14.2	16.6	19.1	19.5	18.6	18.7	17.78
saturated	15.8	15.3	19.3	21.2	25.0	24.8	20.23
mono-unsaturated	25.2	26.8	25.5	24.2	23.0	25.3	25.00
PUFAs	59.0	57.9	55.2	54.6	52.0	49.9	54.77
Unit by the Sun + LEDs							
% lipids in DM	17.4	19.8	23.1	24.4	20.6	19.7	20.83
saturated	21.5	17.6	16.3	15.5	12.7	12.7	16.05
mono-unsaturated	22.3	23.7	22.0	19.1	26.6	33.3	24.50
PUFAs	56.2	58.7	61.7	65.4	60.7	54.0	59.45

Table 3
Fatty acid content (% of total FA) in the biomass of the alga *Neocystis mucosa* in platforms illuminated only by the Sun and with night-time illumination by LED sources in the year 2021. *Bracteacoccus bullatus* after Lukavský et al. (2023) cultivated in a pilot-plant unit, and *Chodatodesmus australis* after Řezanka et al. (2021), cultivated in the laboratory under a temperature <10 °C and continuous irradiation from LEDs of 65 μmol.m^{-2}.s^{-1}.

The Sun	Abbreviations	5.5.21	10.5.21	24.5.21	36.21	10.6.21	*Bracteoc.*
16:0	Palmitic	14.5	13.8	17.3	19.0	23.2	15.6
16:1ω-7	Palmitoleic	3.6	4.1	4.4	4.3	3.8	3.1
16:1ω-9		1.0	1.2	1.4	1.1	1.0	1.2
16:2ω-6	Hexadecadienoic	4.4	5.0	5.3	5.2	4.8	6.0
16:3ω-3	Hexadecatrienoic	4.8	5.2	5.0	5.6	4.9	5.2
16:4ω-3		5.7	5.6	4.8	3.8	4.0	5.1
18:0	Stearic	1.3	1.5	2.0	2.2	1.8	2.1
18:1ω-9	Oleic	18.6	19.4	17.5	17.1	16.6	22.6
18:1ω-7	cis-Vaccenic	2.0	2.1	2.2	1.7	1.6	2.4
18:2ω-6	Linoleic	21.5	21.0	19.8	20.0	18.6	18.3
18:3ω-6	γ-Linolenic	0.5	0.4	0.4	0.3	0.3	0.4
18:3ω-3	α-Linolenic	21.5	19.7	19.0	18.6	18.5	17.4
18:4ω-3	Stearidonic	0.6	1.0	0.9	1.1	0.9	0.6

S + LEDs	Abbreviations	5.5.21	10.5.21	24.5.21	3.6.21	10.6.21	*Chodatod.*
16:0	Palmitic	19.7	15.4	14.2	13.1	10.7	6.4–10.1
16:1ω-7	Palmitoleic	4.7	3.6	4.0	4.2	4.1	–
16:1ω-9		0.6	0.8	1.0	1.2	1.5	3.6–6.2
16:2ω-6	Hexadecadienoic	4.1	5.7	6.5	6.1	5.7	–
16:3ω-3	Hexadecatrienoic	4.4	5.1	5.6	7.2	6.6	3.3–5.6
16:4ω-3		4.5	5.0	5.7	5.6	5.7	10.9–19.2
18:0	Stearic	1.8	2.2	2.1	2.4	2.0	0.9–1.4
18:1ω-9	Oleic	15.2	17.0	14.7	11.7	19.2	15.6–27.5
18:1ω-7	cis-Vaccenic	1.8	2.3	2.3	2.0	1.8	0.5–1.1
18:2ω-6	Linoleic	19.1	22.3	23.8	24.6	23.2	16.5–20.3
18:3ω-6	γ-Linolenic	0.3	0.4	0.5	0.3	0.2	–
18:3ω-3	α-Linolenic	23.4	19.3	18.5	19.8	18.4	19.7–25.3
18:4ω-3	Stearidonic	0.4	0.9	1.1	1.8	0.9	3.0–5.8

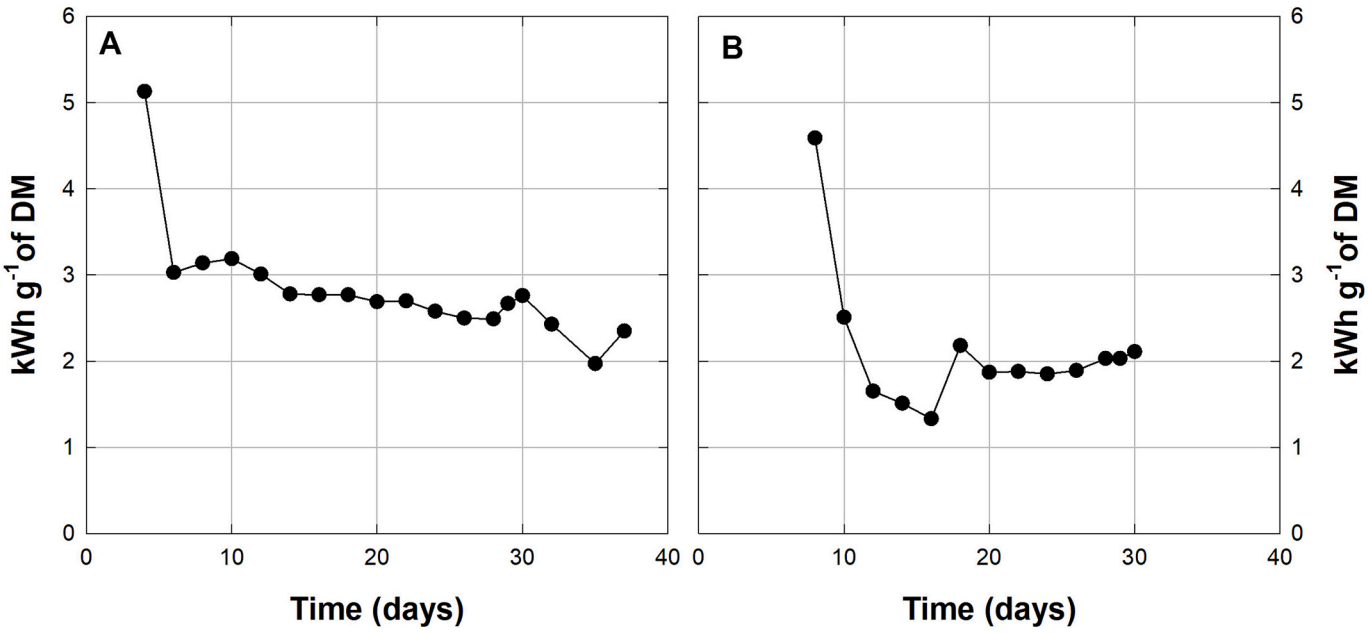

Fig. 6. Consumption of kWh per gram of dry matter for a unit with night illumination by LEDs, in the pilot-scale cultivation of the alga *Neocystis mucosa*. Time courses of dependencies of electricity consumption in times of cultivation, 10.1.-11.2.2022 (A), 14.1.-6.2.2022 (B). Dry matter was evaluated gravimetrically in 2 day intervals.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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