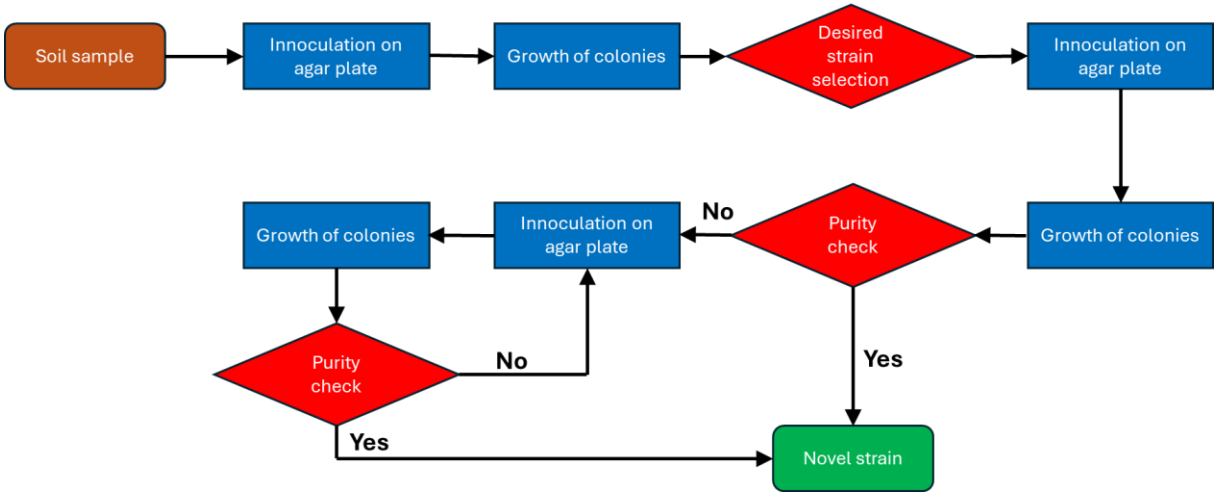
Detailed strain isolation protocol according to Andersen (2005):

The soil sample was resuspended in 0.5 ml of sterile liquid ½ BG-11 medium (Allen 1968, Allen and Stainer 1968, Rippka et al. 1979, Andersen 2005) was spread evenly on the agar plate containing ½ BG-11 medium (Allen 1968, Allen and Stainer 1968, Rippka et al. 1979,) solidified by 2 % agar. The algal colonies were grown in a cultivation box at temperature of 12 °C and irradiance of 30 µmol m^{-2} s^{-1}. Then, the individual colonies were identified under the light microscopy using the following keys and manuals: (Růžička, 1977,1981; Ettl, 1978; Komárek& Fott, 1983; Krammer & Lange-Bertalot, 1986, 1991a, 1991b; Komárek & Anagnostidis, 1998, 2005; Komárek, 2013). The selected colony was transferred by microbial loop to a new agar plate and was streaked over the agar plate. The culture was let to grow in the same conditions as previously. New colonies, arising from individual cells, were evaluated by light microscope for fungal contamination or presence of other algae or cyanobacteria. Colony with the desired alga was transferred to a new agar plate, streaked and the culture was let to grow in in the same conditions as previously. This procedure was repeated until unialgal culture was obtained.

Isolation process scheme



BG-11 medium recipe according to Allen 1968, Allen and Stainer 1968, Rippka et al. 1979

Into 900 ml of dH_2O add 1 ml of the Fe-citrate solution, and then add remaining components. Autoclave. Final pH should be 7.4 after cooling.

Component	Stock Solution (g l^{-1} dH_2O)	Quantity Used (l^{-1} dH_2O)
Fe-citrate solution	see following recipe	1 ml
$NaNO_3$	-	1,5 g
$K_2HPO_4.3H_3O$	40	1 ml
$MgSO_4.7H_2O$	75	1 ml
$CaCl_2.2H_2O$	35	1 ml
Na_2CO^3	20	1 ml
Na-EDTA	1.0	1 ml
Trace metals solution	see following recipe	1 ml

Fe-citrate solution

Component	Stock Solution (g l^{-1} dH_2O)	Quantity Used (l^{-1} dH_2O)
Citric acid	6	1 ml
Ferric ammonium citrate	6	1 ml

Trace metals solution

Component	Stock Solution (g l^{-1} dH_2O)	Quantity Used (l^{-1} dH_2O)
H3BO3	-	2,860 g
MnCl2.4H2O	-	1,810 g
ZnSO4.7H2O	-	0,220 g
CuSO4.5H2O	79,0	1 ml
Na2MoO4.2H2O	-	0,391 g
Co(NO3)2.6H2O	49,4	1 ml

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Table 1 Supplement The summary of microenvironmental conditions during *Neocystis mucosa* cultivation in LED + Sun and Sun pilot plants. The statistically significant differences (paired t-test for period when both pilot plants were in operation) are marked in bold. Abbreviation: PAR – photosynthetically active radiation, s.d. – standard deviation.

	2021 (spring – summer)		Stat.significance		**2022 (winter – spring)**				Stat.significance			
	LED + Sun	**Sun**			**LED + Sun**			**Sun**	Batch 1 × Sun		Batch 2 × Sun	
			t-value	P-value	Both batches	Batch 1	Batch 2		t-value	P-value	t-value	P-value
Start cultivation (CET)	16.5. 8:00	21.5. 11:00	21.5. 11:00		10.1. 9:20	10.1. 9:20	11.2. 7:00	10.1. 9:20	10.1. 9:20		11.2. 7:00	
End cultivation (CET)	23.6. 6:00	28.6. 9:00	23.6. 6:00		14.4. 7:00	11.2. 7:00	14.4. 7:00	28.3. 7:00	11.2. 7:00		28.3. 7:00	
Cultivation duration (d)	38	38	33		94	32	62	77	32		45	
Cultivation conditions												
number of cases (max)	*5476*	*5463*	*4738*		*13523*	*4594*	*8929*	*11075*	*4594*		*6481*	
Air temperature												
Mean ± s.d. (°C)	**26.4 ± 5.5**	**23.5 ± 7.4**	**61.89**	**<0.001**	**17.0 ± 6.0**	**15.1 ± 3.4**	**18.1 ± 6.8**	**12.2 ± 5.8**	**98.73**	**< 0.001**	**98.98**	**< 0.001**
Median (°C)	26.0	23.1			16.5	15.9	17.1	9.6				
Minimum (°C)	11.7	9.0			6.7	7.5	6.7	3.7				
Maximum (°C)	41.0	41.6			41.5	30.2	41.5	39.1				
Suspension temperature												
Mean (°C)	**23.3 ± 4.6**	**21.4 ± 5.6**	**65.67**	**<0.001**	**14.1 ± 4.6**	**12.3 ± 2.3**	**15.0 ± 5.1**	**10.9 ± 4.5**	**103.0**	**< 0.001**	**100.0**	**< 0.001**
Median (°C)	23.0	21.2			14.4	12.3	13.8	8.6				
Minimum (°C)	11.0	9.1			6.5	7.3	6.5	4.1				
Maximum (°C)	36.2	38.2			32.4	23.3	32.4	28.2				
Irradiance (PAR)												
Mean (µmol m^{-2} s^{-1})	**389 ± 303**	**199 ± 250**	**30.62**	**<0.001**	**438 ± 390**	**552 ± 399**	**379 ± 372**	**64 ± 119**	**81.51**	**< 0.001**	**71.62**	**< 0.001**
Median (µmol m^{-2} s^{-1})	272	71			272	552	197	0				
Minimum (µmol m^{-2} s^{-1})	3	0			0	0	0	0				
Maximum (µmol m^{-2} s^{-1})	1438	1332			1098	935	1098	1611				
Diel sum of PAR												

Mean (MJ m^{-2} d^{-1})	**7.18 ± 1.08**	**3.53 ± 1.15**	**21.4**	**<0.001**	**8.10 ± 3.99**	**10.08 ± 2.73**	**7.06 ± 4.17**	**1.00 ± 0.66**	**20.64**	**< 0.001**	**14.01**	**< 0.001**
Median (MJ m^{-2} d^{-1})	7.40	3.44			10.30	11.04	9.86	0.87				
Minimum (MJ m^{-2} d^{-1})	2.75	0.83			0.10	0.50	0.10	0.02				
Maximum (MJ m^{-2} d^{-1})	8.57	5.10			11.89	11.89	10.75	2.67				
Total sum of PAR during cultivation (MJ.m^{-2})	280.01	137.66			777.60	332.60	445.00	77.86				

Table 2 Supplement Abbreviations of fatty acids evaluated in biomass of the alga.

short designation	abbre-viation	trivial name	systematic name
16:0	PA	palmitic acid	hexadecanoic acid
16:1ω-7	PO	palmitoleic acid	9-cis-hexadecenoic acid
16:1ω-9	HA	hypogeic acid	7-cis-hexadecenoic acid
16:2ω-6			7-cis,10-cis-hexadecadienoic acid
16:3ω-3	RA	roughanic acid	7-cis,10-cis,13-cis-hexadecatrienoic acid
16:4ω-3			4-cis,7-cis,10-cis,13-cis-hexadecatetraenoic acid
18:0	S	stearic acid	octadecanoic acid
18:1ω-9	OL	oleic acid	9-cis-octadecenoic
18:1ω-7	VA	cis-vaccenic acid	11-cis-octadecenoic
18:2ω-6	LA	linoleic acid	9-cis,12-cis-octadecadienoic acid
18:3ω-6	GLA	γ-linolenic acid	6-cis,9-cis,12-cis-octadecatrienoic acid
18:3ω-3	ALA	α-linolenic acid	9-cis,12-cis,15-cis-octadecatrienoic acid
18:4ω-3	ST	stearidonic acid	6-cis,9-cis,12-cis,15-cis-octadecatetraenoic acid

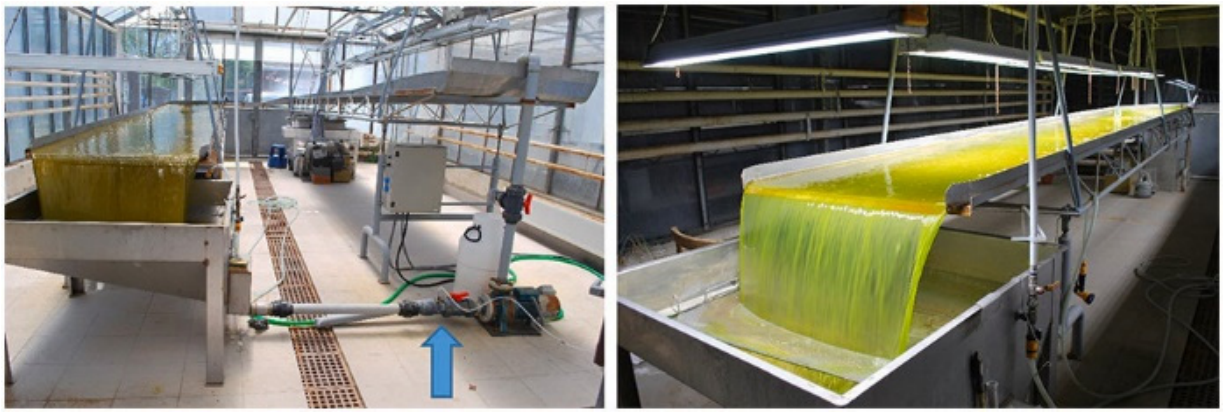

Fig. 1 Supplement. Pilot plant cultivation of the alga *Neocystis mucosa* on a thin-layer platform, with volume of 150 L and area of 12 m^2. Unit with additional LED lighting, during day (left) and during night. LEDs are switched on and off in light of 20 $\mu mol.m^{-2}.s^{-1}$. CO_2 is inserted before pump (arrow).

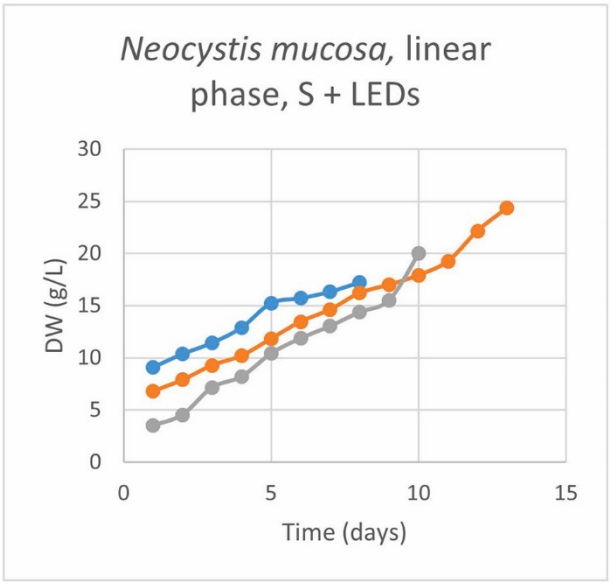

Fig. 2 Supplement. The comparison of the linear phases of growth curves of alga *Neocystis mucosa* during semi-continuous cultivation, 3 repetitions (1st, 2nd and 3rd harvesting), cultivation with night illumination, in semi-continuous mode (see Fig. 5). The slopes of the growth curves are practically identical.

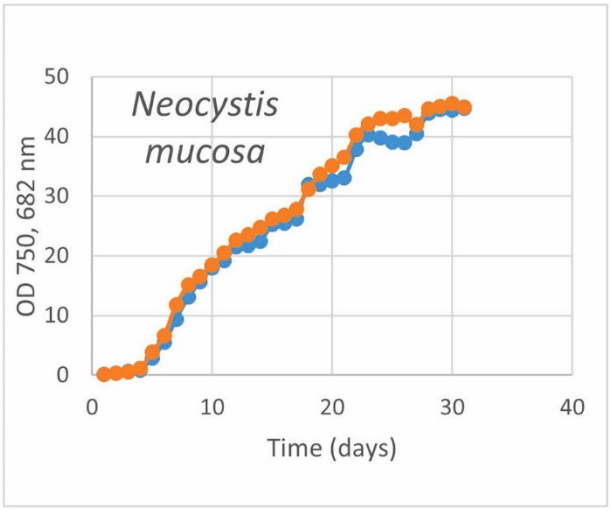

Fig. 3 Supplement. Semi-continuous cultivation of the alga *Neocystis mucosa* evaluated as OD 750 (orange curve) and 682 nm (blue curve), in a unit irradiated by the Sun during days and by LEDs during nights. Cultivation 16.5. - 15.6.2021.

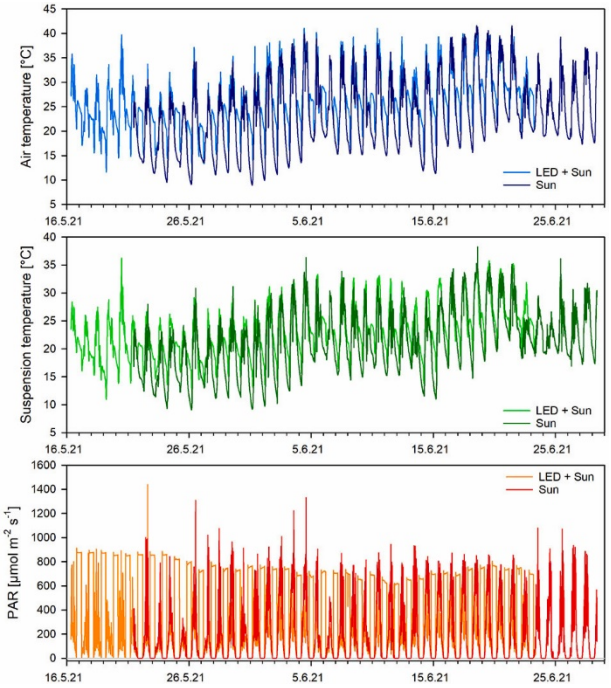

Fig. 4 Supplement. Temperature and irradiance (PAR – photosynthetically active radiation) values recorded by dataloggers during the cultivation of the green alga *Neocystis mucosa*, in pilot-plant units, when illuminated only by the Sun and when illuminated at night by LED sources during spring-summer 2021. Statistical summary of the microenvironmental conditions are shown in Table 1 Supplement.

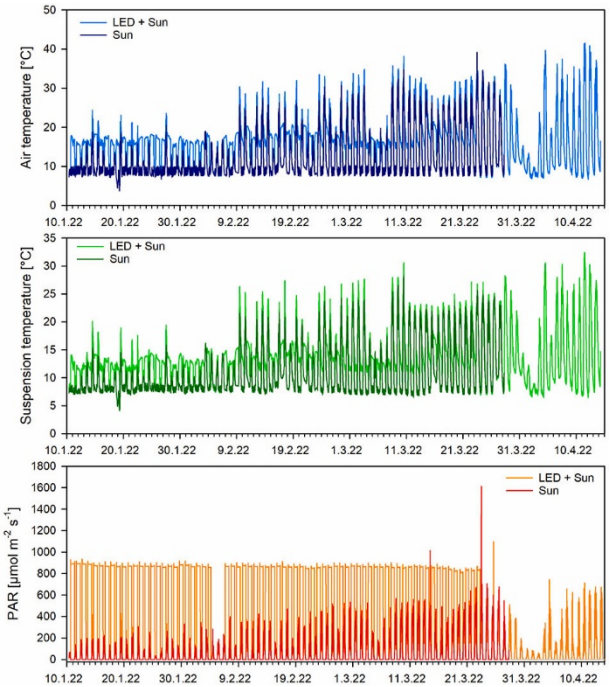

Fig. 5 Supplement. Temperature and irradiance (PAR – photosynthetically active radiation) values recorded by dataloggers during the cultivation of the green alga *Neocystis mucosa*, in pilot-plant units, when illuminated only by the Sun and when illuminated at night by LED sources during winter-spring 2022. Statistical summary of the microenvironmental conditions are shown in Table 1 Supplement.