



Neochloris oleoabundans oil production in an outdoor tubular photobioreactor at pilot scale

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Abstract

Oil production was tested with *Neochloris oleoabundans* in a 6 m³, horizontal soft sleeve tubular reactor from 22 October to 7 November in Matalascañas, southern Spain. Biomass productivity during the nitrogen replete phase was 7.4 g dw m⁻² day⁻¹. Maximum lipid content in the biomass was 39% and average lipid productivity during the nitrogen depletion phase was 2.0 g m⁻² day⁻¹. Nitrogen depletion of the cultures was carried out in order to enhance fatty acid formation, using the inverse nitrogen quota in the biomass to predict the fatty acid content. TFA concentration at harvest was 14% DW, compared to a value of 17%, predicted by the inverse nitrogen quota. The overall feasibility of the horizontal tubular technology for microalgal oil production, including mixing energy expenditure, was evaluated.

Keywords Oil · Fatty acids · Productivity · *Neochloris oleoabundans* · Oil enhancement

Introduction

Microalgal oil production for transportation fuels received considerable interest during a period with high crude oil prices, roughly from 2005 to 2014 and multiple projects were started to develop microalgal production and downstream processing technology. We carried out a full-scale projection study of various algal biomass production techniques in (Norsker et al. 2011) and we examined photobioreactor auxiliary energy requirements in (Norsker et al. 2012).

We concluded that the horizontal tubular photobioreactor with disposable soft sleeve-tubes had the best development potential to become a full-scale production technology for microalgal transportation fuels. Tubular photobioreactors are

used both for full scale biomass production and to produce starter cultures for open pond production of the algal biomass.

In recent years, a number of tubular photobioreactor production plants have been established (Fernández et al. 2020). Tube volume of the largest of these exceeds 1000 m³. The plants, however, produce high-value products, such as food supplements while microalgae are not yet to any significant extent used as a feedstock for biofuels production and most of the algal fuel projects have either been closed down or changed focus to high value products. Nevertheless, a number of oil companies are running projects with the aim to develop microalgae for biodiesel or aviation fuels.

Microalgal oils have a wide range of other applications, such as food and feed, raw materials for chemical and pharmaceutical industry, and cosmetics so developing oil production with microalgae is still relevant.

One of the challenges in developing oil production with microalgae is monitoring algal oil content. The oil content can be quantified as total lipids and TFA (total fatty acids). The lipid extract contains chlorophyll, carbohydrate moieties of glycolipids, and other non-oil pigments. Lipid content in microalgae ranges from 5 to over 80% dry weight (dw), according to an extensive review of Qiang, considering all strains and growth conditions (Qiang et al. 2008).

Total fatty acids (TFA) are the most relevant biofuel measure when considering extraction as fatty acid methyl esters (FAME). The FAME fraction of lipid extracts depends on

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lipid composition because TAGs (triglycerides) convert 100% to FAME while for example the glycolipid di-galactosyl diglyceride, converts to only 63% FAME (Laurens et al. 2012). Discrepancy between lipid and TFA content may therefore be considerable.

The production of microalgal oils generally follows a two-phase pattern with the main biomass production taking place in a nutrient replete stage while enhancement of oil content may follow in a nutrient depleted phase, during which biomass production is reduced or even may become negative.

Oil content enhancement normally is done in a batch process. Continuous fatty acid production technology has also been proposed; with *Neochloris oleoabundans*, TFA productivity from 60 to 118 mg L⁻¹ day⁻¹ was obtained with varying degree of nitrogen limitation; the high value obtained with nitrogen replete cultures (Klok et al. 2013) but continuous production appears not as efficient as batch technology in terms of volumetric productivity; in a direct comparison of cultivation technology, batch TAG productivity was 230 mg L⁻¹ day⁻¹ in nitrogen limited *Acutodesmus obliquus* as opposed to 110 mg L⁻¹ day⁻¹ in continuous cultivation. The difference appeared to be related to higher light utilization efficiency with the batch compared to continuous culture (Remmers et al. 2017).

The term *light utilization efficiency* or yield on light (in g lipid/TAG/TFA per mol photons (PAR)) is particularly useful for characterizing a given laboratory or field reactor result in terms of what potentially is possible. Remmers and coworkers suggested the maximum realistic TAG yield on light being about 0.53 g mol⁻¹photons, taking TAG content as 50% dry biomass (Remmers et al. 2018). Values of 0.32 g TAG mol⁻¹photons were found in lab reactors for low-light cultivated *Chlorella zofingiensis* at the onset of nitrogen starvation (Mulders et al. 2014). In outdoor flat- and stacked horizontal tubular reactors, TAG yield on light varied between 0.07 and 0.19 g mol⁻¹photons for *Nannochloropsis sp.* (Benvenuti et al. 2015).

Recently, a minimum irradiation has been also shown necessary to support TAG yield in *Nannochloropsis gaditana* under nitrogen limitation (Janssen et al. 2018). TAG yield on light by *N. gaditana* was maximal (0.09 g mol⁻¹photons) at intermediate biomass *specific irradiation*, ranging between 11 and 26 μmol photons s⁻¹ g⁻¹ (Janssen 2018). The reported productivities are not long-term sustainable, but typically apply to nutrient limited batch cultures over 4–7 days, after which time productivity declines. With outdoor cultures however, it is difficult to implement such knowledge to optimize oil productivity because of dynamic irradiation, both in terms of day-night cycles and cloudiness.

In a tubular photobioreactor, photosynthesis results in an accumulation of dissolved oxygen during the passage of the culture from one degassing event to the next. Excessive dissolved oxygen exposure may lead to photo inhibition and tube

length is therefore limited by the acceptable dissolved oxygen concentration build-up over the tube length. Maximum tube length can be calculated as a function of acceptable oxygen concentration at the end of the tube, photosynthetic rate and fluid velocity and Fernández et al. applied a tube length of 80 m (Ación Fernández et al. 2001).

Photoinhibition of an outdoor algal culture is a risk. Photosynthesis in a microalgal culture may be considered a function of average irradiance (Fernández et al. 2020) and photoinhibition may consequently be considered to result if a culture is exposed for extended time to supersaturating average irradiance levels. Starting the photobioreactor with low biomass density may thus result in the culture becoming photo inhibited, possibly irreversibly. It is difficult to find any systematic approach in the literature as to how to start the photobioreactor and to what extent the minimum starter culture concentration depends on strain, state of light adaptation and climatic conditions. A few general recommendations can be found, including scaling up in steps of 5–10 times, not exposing the culture to full sunlight during the first days and ensure biomass densities between 5 and 10 g dw m⁻² (Sergejevová et al. 2015).

Nutrient supply is critical for the result of the fatty acid enhancement procedure. With prolonged nitrogen starvation, high oil content can be obtained but oil productivity tends to be low because of low biomass productivity. With higher nitrogen supply, oil productivity may be high but at high biomass productivity and low oil content. The overall industrial optimum lies between these two extremes (Adams et al. 2013) and depends on strains characteristics, cultivation conditions, dewatering- and oil extraction technique. Determining the optimum oil content in the algae is therefore arguably complex.

The current work describes the pilot-scale test of microalgal oil production in an outdoor, horizontal tubular photobioreactor in the South of Spain. Matalascañas in the Huelva region in south-west Spain was selected because of low cloudiness which results in monthly average daily irradiation most of the year between 20 and 25 MJ m⁻² day⁻¹, peaking on some summer days around 30 MJ m⁻² day⁻¹. In December and January, average daily irradiation is around 10 MJ m⁻² day⁻¹. Summer temperatures rarely exceed 35 degrees. The objective was, besides gaining experience with the cultivation technology, to test whether an oil content of 30% dw could be achieved with the selected strain, measured as total fatty acid fraction of biomass.

Materials and methods

Strains

Neochloris oleoabundans (UTEX 1185) was maintained Bold's basal medium (Pruvost et al. 2009). *Chlorococcum*

littorale (NBRC 102761) was maintained in Zou medium (Zou and Richmond 1999). Cultures were maintained in 250 mL E-flasks in an illuminated (25 μmol photons m⁻² s⁻¹) orbital shaker with air with 5% CO₂ and temperature control (25 °C).

Analyses

Lipids For total lipids content measurement, biomass was recovered and washed with 0.5 M ammonium formate and oven dried at 60 °C. The biomass was ground in a mortar and homogenized by sonication in chloroform-methanol. Total lipids were extracted in an automated Soxhlet device as described in Ruiz-Domínguez et al. (2015).

Fatty acids were measured in a modified Bligh & Dyer analysis (Breuer et al. 2013). For the tubular photobioreactor experiments, methylated lipid extracts, produced at the test site in Matalascañas, were sent to Wageningen University and analyzed further, according to Breuer et al. (2013) Internal standards for all 6 identified fatty acids were included.

Lipid productivity, Pl_n or total fatty acid productivity, $Ptfa_n$ on the n 'th day during the final batch phase was calculated according to:

$$Pl_n = \left((Cb_n * Xl_n) - (Cb_0 * Xl_0) \right) / n \quad [g L^{-1} day^{-1}] \quad (1)$$

$$Ptfa_n = \left((Cb_n * Xtfa_n) - (Cb_0 * Xtfa_0) \right) / n \quad [g L^{-1} day^{-1}] \quad (2)$$

with Cb_n being biomass concentration in biomass on day n , Cb_0 being biomass concentration in the reactor at the first day of lipid- or total fatty acid sampling, Xl_n and $Xtfa_n$ being lipid fraction and total fatty acid fraction of biomass at day n , respective. Xl_0 and $Xtfa_0$ being lipid- and total fatty acid fraction of biomass at the first day of sampling respective and n the day number after the beginning of the lipid sampling.

Biomass Biomass dry weight was measured in triplicate on pre-dried GF/F fiberglass filters, washing the filters with 0.5 M ammonium formate before drying 24 h at 95 °C.

Nitrate and phosphate Samples for nitrate, phosphate, lipid, and fatty acid content were taken daily, around noon. Nitrate and phosphate were analyzed in a continuous flow analyzer (Proxima, Alliance). Nitrate was determined colorimetrically after reaction with sulfanilamide and 1-naphtyl ethylenediamine as a difference between values before and after nitrate reduction over cadmium. Range 48–161 nM. Phosphate was determined colorimetrically after reaction with ammonium heptamolybdate and potassium antimony oxide tartrate. Range 6–323 nM.

Irradiation Irradiation measurements were kindly provided by the meteorological station at INTA (Instituto Nacional de Técnicas Aeroespaciales), belonging to the Spanish Ministry of Defense, situated at Arenosillo, about 25 km from the experimental research station in Matalascañas. Data used were hourly accumulated global horizontal irradiation, I_{acc} [in kJ m⁻² h⁻¹].

These values were converted to hourly average horizontal irradiance (PAR), H_{avg} [μmol photons m⁻² s⁻¹], using the formula in Eq. 3:

$$H_{avg} = [\mu mol photons s^{-1} m^{-2}] \\ = 4.6 * 0.43 * \frac{1}{3.6} * I_{acc} \quad [kJ m^{-2} h^{-1}] \quad (3)$$

with the factor 4.6 denoting the average solar photon number per joule; 0.43 denotes the PAR ratio to full solar spectrum and the factor 1/3.6 combines the conversion from hours to seconds and kJ to J (Slegers et al. 2011).

I_{av} , [μmol photons m⁻² s⁻¹] is the average irradiance inside the tubes. I_{av} was calculated with the integrated Lambert-Beer formula (Eq. 4). I_0 [μmol_{PAR} photons m⁻² s⁻¹], the irradiation impinging on the tube surface, was taken as the irradiation on the horizontal projection of the tubes, p [m], the photic depth was taken as the tube diameter. C_b [kg dw m⁻³] is biomass concentration and K_a is absorption coefficient for the algal biomass.

$$I_{av} = \frac{I_0}{p * C_b * K_a} (1 - \exp(-p * C_b * K_a)) \quad [\mu mol_{PAR} photons m^{-2} s^{-1}] \quad (4)$$

Total irradiation impinging on the reactor per day was calculated by summing I_{acc} over the hours of the day, omitting the time in the evening, where the reactor was completely shadowed by an adjacent laboratory building and measured irradiance was 0–200 μmol photons m⁻² s⁻¹. Total impinging irradiation constituted 77–94% of the total horizontal irradiation.

Photosynthetic efficiency, PE is the ratio of biomass energy produced during a growth period to the total solar light energy assimilated by the culture during the period.

In the actual context, the nutrient replete growth rate, $\frac{\Delta C_b}{\Delta t}$ was multiplied with the estimated calorific content (ΔH_c) and divided by $IR_{avg,t}^{t+\Delta t}$, the average horizontal irradiation during the period. This is a simplification, assuming that the light absorption can be taken as the horizontal irradiation, impinging on projection of the tube. Also, it should be noted that the calorific content, ΔH_c was not measured but estimated on the

basis of literature values (Table 1) to be characteristic for nutrient replete *Neochloris* biomass.

$$PE = \frac{\Delta Cb}{\Delta t} * \frac{\Delta H_c}{IR_{avg_t}^{t+\Delta t}} \quad (5)$$

The calorific content value used in the calculation of photosynthetic efficiency during the nutrient replete phase was 21 kJ g⁻¹. To justify the used value, some literature values for algal biomass calorific values ΔH_c are presented in Table 1. Unfortunately, there are no published values on calorific content of nutrient replete *Neochloris*, but a figure in the low range of values for *Chlorella* would be reasonable to use.

Growth medium

A concentrated and storage-stable medium solution was designed for addition to natural or artificial sea water in order to make growth medium for high biomass density cultures of marine algae at large scale. The composition of the ready-made medium (accounting only for the components in the nutrient solution) is given in Table 2. The complete recipe of the nutrient solution is given in Norsker et al. (2021).

Sodium, calcium, and magnesium are excluded from the medium solution, which means that it is long-term stable (non-precipitating). The recipe had been tested with a batch culture of *N. oleoabundans* and sufficed for a biomass concentration of 7 g dw L⁻¹.

In the large-scale nutrient replete algal production, biomass concentration was kept below 3 g dw L⁻¹.

For introduction of nitrogen limitation in the cultivation, the NaNO₃ was reduced without modifying the other medium components.

Inverse nitrogen quota

For *Neochloris*, the relation between fatty acid content and inverse nitrogen quota could be described according to the formula:

Table 1 Calorific content values from literature

Source	Values (kJ g ⁻¹)	Reference
Various literature references	20–23.75	(Weyer et al. 2010)
Oil rich (lipid 50% dw) biomass	26.9	(Weyer et al. 2010)
<i>Chlorella vulgaris</i> , 58% lipid	28	(Scragg et al. 2002)
<i>Chlorella vulgaris</i> , 28% lipid	21	(Scragg et al. 2002)
<i>Chlorella</i> , axenic, lab batch	22	(Halfhide et al. 2014)

Table 2 Composition of the medium, used to propagate and produce the microalgal species in the study

Mineral	Concentration
NaNO ₃	50 mM
KH ₂ PO ₄	1.7 mM
Na ₂ EDTA	173 μM
FeSO ₄	108 μM
MnCl ₂	11 μM
ZnSO ₄	2.3 μM
Co(NO ₃) ₂	0.24 μM
CuSO ₄	0.1 μM
Na ₂ MoO ₄	1.1 μM
NaNO ₃	50 mM
KH ₂ PO ₄	1.7 mM
Na ₂ EDTA	173 μM

$$y = 0.4807 * x^2 + 0.0893 * x + 0.1107 \quad R^2 = 0.79 \quad (6)$$

with y being total fatty acid content in biomass (TFA% dw) and x being the inverse nitrogen quota in g dw mmol⁻¹N (Norsker et al. 2021). 30% TFA concentration in biomass was set as a target and a total of 31.1 mol nitrogen had been used to produce the batch, including nitrogen added in the propagation step of the biomass as the biomass of the propagation reactor and the 600 L reactor was transferred as a total. Using the formula, the target inverse nitrogen quota of 0.54 g dw mmol⁻¹N could be calculated which would require a final biomass concentration of 2.73 g dw L⁻¹.

Biomass propagation

Propagation was done in an 8-panel, semi-continuously operated reactor (see Fig. 1). The dimensions of the panels were 50 × 50 × 1 cm, filled to a volume of about 1.7 L. The panels were made by Proviron (Belgium) from clear polypropylene film and front- and back sides were welded together creating vertical channels. Each channel was sparged from a chamber below the channels. The vertical channels were constructed identically, i.e., not as either air-lifts or downcomers. The panels were suspended on both sides of a bank of 20 fluorescent T5 tubes (54 W) with electronic ballasts at a distance of approximately 20 cm from the panels. Maximum irradiance at the surface of the panels was 450 μmol photons m⁻² s⁻¹, normally operated at 200–300 μmol photons m⁻² s⁻¹.

A diaphragm pump (500 W) recycled the aeration gas. A fixed flow of CO₂ and compressed air from mass flow controllers was sufficient to control pH.

About 25% of the culture was exchanged daily with fresh medium. In total, about 3–4 L was harvested daily at a biomass concentration of 2–4 g dw L⁻¹ and stored in the dark at

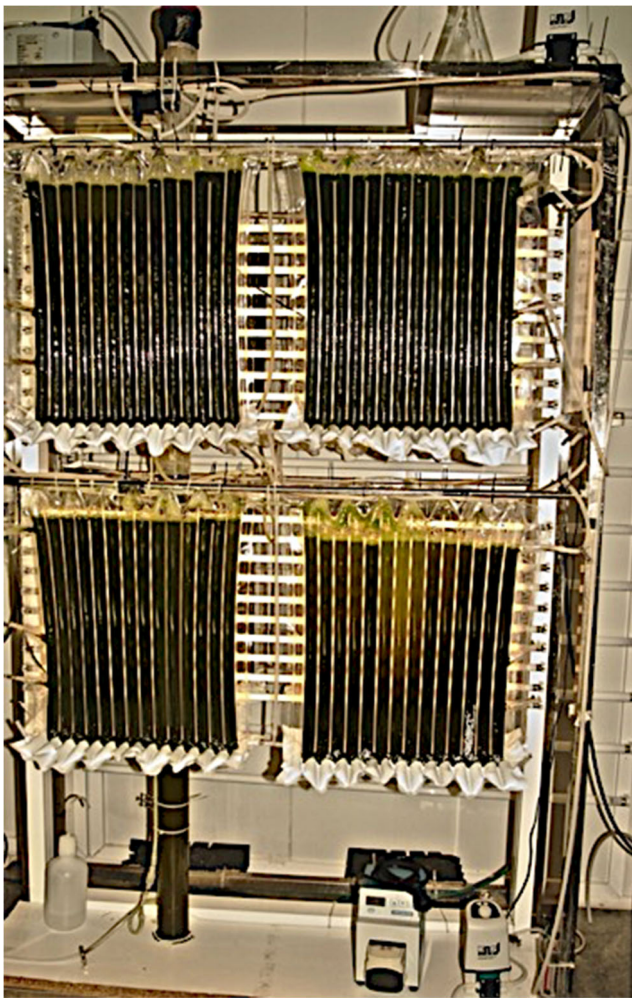


Fig. 1 Propagation reactor with 8 ProviApt panels (4 of them behind fluorescent tube banks)

4 °C until use. Cultures typically retained their productivity for 2–3 weeks and cleaning would then be required to remove biofilm accumulation. The reactor was operated aseptically and unialgal cultures were produced.

The 600 L-photobioreactor

The 600-L photobioreactor is a soft-sleeve tubular photobioreactor with a single, 24 m long lane and a single degasser-buffer tank. It was located adjacent to the 6m³ reactor, near the fence (Fig. 2). The manifold system was similar to the 6 m³ reactor. The circulation pump was a 2.3 kW ESPA, 3 × 380 V, 2650 rpm swimming pool pump. Normally, the ESPA pump was operated at a power supply of 0.90 kW m⁻³, effectively subjecting the biomass to a much higher shear stress than the circulation pump in the 600 L reactor. Degassing was carried out by falling film: in the degasser-buffer tank, the inlet ejected vertically up-wards with a 75 mm tube, falling down as an umbrella-shaped sheet of



Fig. 2 The two out-door photobioreactors on the 27 Nov 2011. In front, the 6 lanes belonging to the 6 m³ reactor; in the back, the single lane of the 600 L reactor, used for producing starter culture for the 6 m³ reactor. The tube crossing the 3 front lanes is the harvest line

fluid. This degassing method was found more effective and energy-economic than air sparging (data not shown).

At the *N. oleoabundans* campaign, reported here, about 50 g dw of biomass was used to start the 600 L reactor, resulting in an initial biomass concentration of 0.1 g dw L⁻¹. To avoid photoinhibition, the tubes initially were covered with white plastic which reduced incident irradiation about 80%. The cover was removed in 3 steps between day 2 and day 3.

The 6 m³ Photobioreactor

A soft-sleeve, tubular photobioreactor was designed by the authors and built by the Dutch engineering company Paques. Tube diameter was chosen as 6 cm in accordance with Acién Fernández et al. (2001). Tube length traveled by the culture between degassing events was 64 m (Cycle of flow in Fig. 3). Disposable polyethylene tubing was used and as the system was designed for batch mode operation, it was not attempted hermetically closed. Instead, it was cleaned and sanitized with 100 ppm sodium hypochlorite for 24 h between campaigns, using short tube length and set up with newly propagated starter culture. Growth medium was made from tap water, natural sea salt, and concentrated nutrient solution and was used unfiltered.

The manifold system consisted of 6 lanes, each with 8 tubes, the 4 central tubes being exit tubes and two peripheral tubes on either side being return tubes. At the end of each lane, all 8 tubes were joined in a manifold. Hard pipe dimensions in the distribution system varied from 200 to 125 mm. The system included a 1 m³ degassing tank with air-spargers and a 1m³ buffer tank before the circulation pump (Figs. 3 and 4). Before the exit main manifold branch, an open, 3-m stand-pipe was inserted (Fig. 4) to avoid the risk of over-pressuring the soft PE tubes. The total volume of the system was 6.1 m³

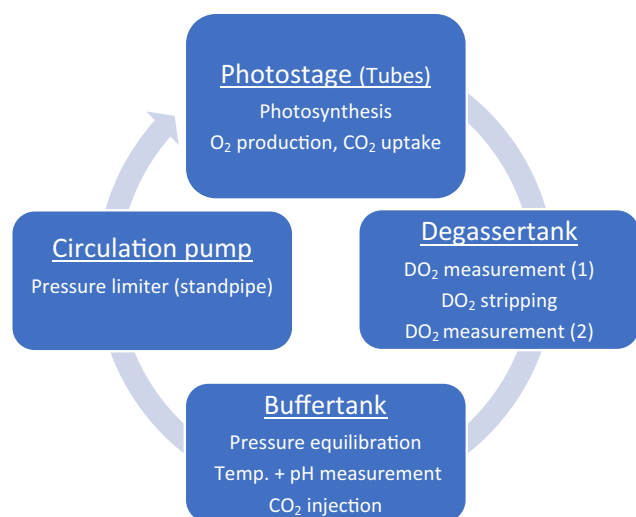


Fig. 3 The photobioreactor process flow diagram

and the horizontally projected tube area was 92 m². Degassing was carried out by air sparging. For more details, see Table 3.

The circulation pump was a KSB kwp K 100–250 with a 224-mm impeller with a design speed of 950 rpm (low shear). The pump was controlled with a frequency converter according to pump speed or power consumption.

Dissolved oxygen was measured at two points—before and after the degassing tank.

pH (used for CO₂ addition control) was measured between the buffer tank and the circulation pump.

Dimensions of the large tubular plant are given in Table 3.

For the 6 m³ reactor, the entire broth from the 600 L culture was used as a starter culture. Inoculation was done in the evening as a precaution to minimize the risk of photoinhibition in the beginning of the cultivation cycle.

Temperature control Due to the late time of year (October/November), temperature control was not necessary as a maximum permissible temperature of 35 °C was found for

Neochloris and the daily temperature maximum only shortly reached that level on the day with the highest irradiation. During summer, temperature was controlled with high pressure water mist spray-nozzles positioned over the tube lanes with 2-me intervals.

pH control pH was controlled at 8.0 with CO₂ injected before the circulation pump to ensure rapid mass transfer of gaseous to dissolved CO₂. pH and dissolved oxygen were measured with a Prosense Qis P915-18 controller with recording of dissolved oxygen, pH, and temperature data.

Flow velocity Flow velocity was measured in one of the 6 outgoing 125 mm exit arms (Fig. 4) with a Sierra model 210 acoustic flowmeter. Flow velocity was set to about 0.45 m s⁻¹ by adjusting the circulation pump frequency controller. The velocity measurement only worked with growth medium, presumably because small gas bubbles accumulate in the medium after algal growth takes on. As a consequence, the frequency controller settings were left constant during the cultivation period. The velocity could therefore have changed during the day as a consequence of foam formation and gas pockets and over the cultivation period as a consequence of biofilm build up.

Biofilm control Nylon beads (PA 10 with a positive buoyancy in sea water) were used during the batch. In the 600 L system, the beads passed perfectly unharmed through the circulation pump; in the 6 m³ reactor system, on the other hand, the beads were gradually macerated during the cultivation cycle. It is uncertain what effect—if any—the beads may have had on the biofilm formation.

Oxygen control Dissolved oxygen content was measured at two points in the plant: before the degassing tank and between

Table 3 Characteristics of the large tubular plant

Photobioreactor dimensions and main operational parameters.	Dimensions
Length of tubes	32 m
Tube length between degassing	64 m
Total projected area of tubing	92 m ²
Total volume of plant	6.1 m ³
Tube diameter	6 cm
Tubing material	PE, UV stabilized
Tubing wall thickness	150 μm
Tube pressure bursting point	0.8 bar
Tube maximum sustained pressure	0.2 bar
Circulation pump flow _{max} at 2.2 kW power supply	900 m ³ h ⁻¹ , 0.4 bar
Air flow (at a point just before pump)	4 m ³ h ⁻¹
Normal flow velocity (at 180 W power supply)	0.45 m s ⁻¹

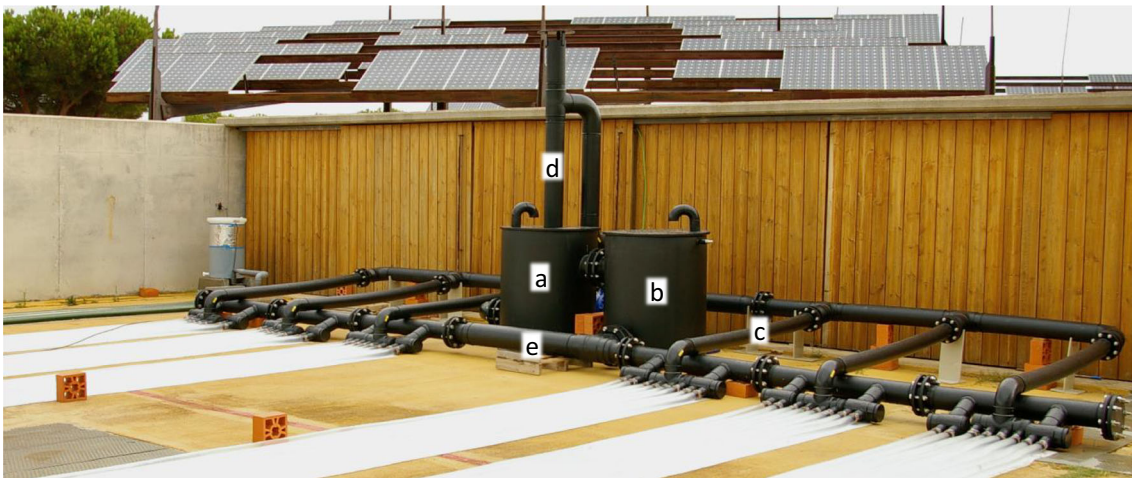


Fig. 4 The photobioreactor flow distribution system

the buffer tank and circulation pump (Figs. 3 and 4). The degassing tank was sparged with atmospheric air from two 60 W diaphragm pumps through 4 sintered, 12 * ø5 cm diffusor cylinders, delivering 4 m³ of air h⁻¹.

Foam and gas pockets Ten parts per million of antifoam Struktol 304 was added manually on demand, normally once per day, to prevent foam formation. Prior to usage in the reactor, antifoam dosage was independently assessed in the laboratory in order to find a suitable concentration which does not compromise algal growth (data not shown). Gas pockets were found to be able to accumulate at high points in the tubes as a result of the unlevelled ground, and it was difficult to evacuate by raising the circulation pump velocity. Instead, gas pockets were removed manually on a daily base with a roller tool that was devised to drive the gas pockets out of the tubes.

Harvest At harvest, the tubes were emptied manually into the buffer tank by lifting the tubes and the culture broth was pumped to a harvest buffer tank from which it was pumped to a GEA KA6 semicontinuous bowl centrifuge. Biomass density obtained with this centrifuge was 21% dw. As the centrifuge could process between 300 and 600 L h⁻¹, depending on algal buoyancy, the harvest procedure lasted 12–24 h from the point the culture circulation was turned off till the biomass was frozen (– 20 °C).

Results

Figure 5 shows that the growth of *N. oleoabundans* in the 600 L reactor followed an exponential rate of 0.34 day⁻¹ without lag phase, suggesting that the irradiation exposure procedure was not excessive as the culture appears to be growing at a constant exponential rate (0.34 day⁻¹) throughout the first 5

to 6 days but also not light limited as a linear growth rate would otherwise be expected.

Furthermore, it should be noted that the biomass had been kept dark at 4 °C for a period of 2 weeks before use and therefore possibly could be more susceptible to irradiation than biomass, acclimated to high irradiance and temperature.

Figure 6 shows the biomass concentration and irradiation of the 6 m³ reactor during the 16 days campaign. The entire culture from the 600 L reactor was used as inoculum for the 6 m³ reactor with initial biomass concentration of 0.141 kg m⁻³.

During the first 10 days, the growth rate is indicated as a linear trend although the first 5 days also could have been suggested to follow an exponential trend but there is too little data to make that conclusion. On day 10, the volumetric productivity dropped from a 0.11 g dw L⁻¹ day⁻¹ to around 0. At

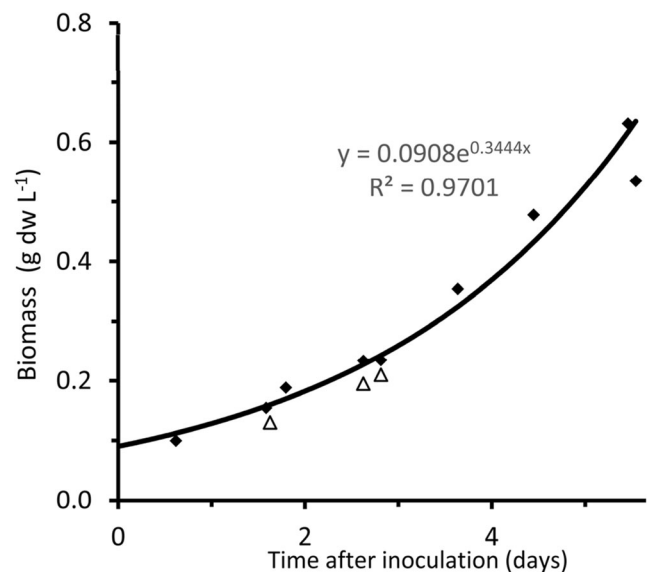
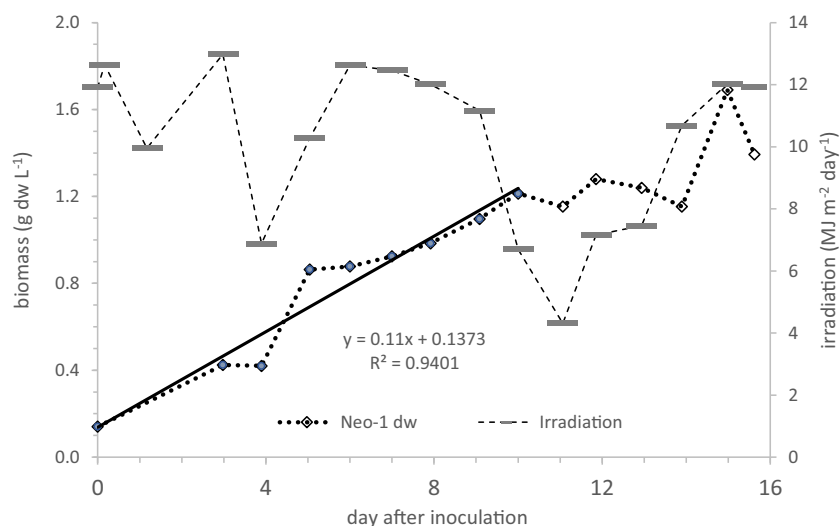


Fig. 5 Biomass growth of *Neochloris oleoabundans* in the 600 L tubular photobioreactor. The three open triangles indicate the time at which the first, second, and last third of the cover over the reactor was removed

Fig. 6 Biomass growth of *Neochloris oleoabundans* and irradiation in the 6 m³ reactor from 22 October to 07 November. Productivity results from this period are summarized in Table 5



day 9, daily irradiation had dropped from a level around 12 MJ m⁻² day⁻¹ to around 4 MJ m⁻² day⁻¹. At that point, residual nitrate concentration was approaching 0 (Fig. 5).

Biomass productivity

During the linear, nutrient replete growth phase from day 0 to day 10 where the residual nitrate concentration was exhausted, volumetric biomass productivity was 0.11 g dw L⁻¹ day⁻¹ (the slope of the linear biomass trend in Fig. 6). This corresponds to an average areal biomass productivity of 7.3 g dw m⁻² day⁻¹ and a photosynthetic efficiency of 1.42% (Table 4). With *C. littorale*, for example, much higher values have been experienced under identical conditions in the present photobioreactor: with *C. littorale*, volumetric productivity values of 0.26 and 0.34 g dw L⁻¹ day⁻¹ were obtained (Figs. 7 and 8, respective) and photosynthetic efficiency values of 4.4 and 4.7% (Tables 4 and 5).

Harvest efficiency

At harvest, which was carried out in a bowl centrifuge, only 52.5% of the biomass that the daily sampling had documented in the reactors was recovered (Table 5). It is unclear what caused the biomass loss during harvest; the centrate appeared visibly clear, but the procedure was lengthy (8 h) during which time the culture was stagnant. Fermentation in the algal biomass or loss of dissolved substances from excretion or cell disruption may have played a role.

Lipid and fatty acid productivity

Average volumetric lipid productivity during the nitrogen limitation period from day 10 to day 16 was 42 mg L⁻¹ day⁻¹ and average areal productivity was 2.0 g m⁻² day⁻¹. Maximum lipid productivities that occurred over the last 2 days (3 datapoints) before the culture was harvested were 149 mg L⁻¹ day⁻¹ and 7 g m⁻² day⁻¹, respectively (Table 5).

Table 4 Initial biomass productivity (nutrient replete) for *Neochloris oleoabundans* and two batches of *Chlorococcum littorale*

	<i>Neochloris oleoabundans</i>	<i>Chlorococcum littorale</i> (1)	<i>Chlorococcum littorale</i> (2)
Period	22.10.2011 to 07.11.2011	10.11.2011 to 18.11. 2011	21.11.2011 to 09.12.2011
Initial volumetric productivity (g dw L ⁻¹ day ⁻¹)	0.11	0.26	0.34
Area productivity (g dw m ⁻² day ⁻¹)	7.3	17.5	22.6
Irradiation in period (MJ m ⁻² day ⁻¹)	10.9	8.3	10.0
Photosynthetic Efficiency (%)	1.4	4.4	4.7

Table 5 Characteristics of the *Neochloris oleoabundans* batch, produced 22.10.2011–07.11.2011 in Matalascañas, south-west Spain. Note that the enthalpy value of 21 kJ g⁻¹ which was used in the photosynthetic efficiency calculation was assumed and not measured

Parameter	Unit	Value
Nitrogen limitation		yes
Date of inoculation		22.10.2011
Date of harvest		07.11.2011
Wet weight harvested	kg	20
Dry weight harvested	kg	4.49
Dry weight produced	kg	8.6
Volumetric biomass productivity (N replete)	g L ⁻¹ day ⁻¹	0.11
Areal biomass productivity (N replete)	g m ⁻² day ⁻¹	7.3
Average irradiation in batch period	MJ m ⁻² day ⁻¹	9.9
Average irradiation lin. growth period	MJ m ⁻² day ⁻¹	10.9
Photosynthetic efficiency	%	1.42
Average volumetric N-deplete lipid productivity (day 10–16)	mg L ⁻¹ day ⁻¹	41.9
Average areal N-deplete lipid productivity (day 10–16)	g m ⁻² day ⁻¹	2.0
Maximum volumetric lipid productivity (day 14–16)	mg L ⁻¹ day ⁻¹	149
Maximum areal lipid productivity, (day 14–16)	g m ⁻² day ⁻¹	7.0
Total period average lipid productivity (day 15)	mg L ⁻¹ day ⁻¹	32
TFA concentration _{max}	% dw	14
TFA concentration _{max, predicted}	% dw	17
Nitrogen addition to reactor	mol	31.1
Target Inverse nitrogen cell quota	g mmol ⁻¹	0.54
Actual final inverse nitrogen cell quota	g mmol ⁻¹	0.28
Biomass specific irradiation	μmol g ⁻¹ s ⁻¹	4.37
Lipid productivity on light	mg lipid mol ⁻¹	120
Density (dw/ww)	%	22.5
Recovery (biomass harvested/biomass produced)	%	52.2

The lipid concentration in the *Neochloris* culture reached 39% dw and TFA reached 14% dw at harvest while the predicted TFA was 17% dw (Fig. 9).

While close to the predicted value, it was far from the originally planned content (30% dw). The reason for

this was that the biomass concentration only reached 1.40 g dw L⁻¹—down from 1.7 on the previous day due to cloudy conditions significantly and lower than the required concentration of 2.73 g L⁻¹ (Fig. 9 and Table 5).

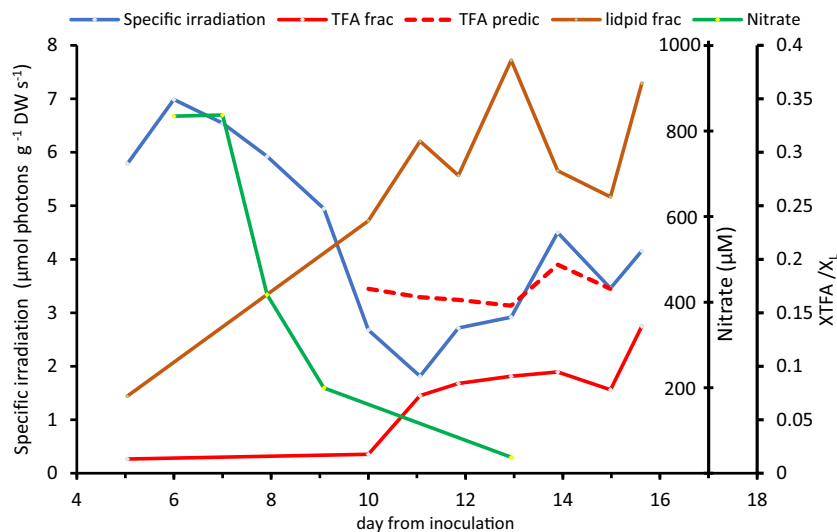


Fig. 7 Biomass growth of *Chlorococcum littorale* (1) in the 6 m³ reactor from 10 November 2011 to 18 November 2011. Productivity results from this period are summarized in Table 5

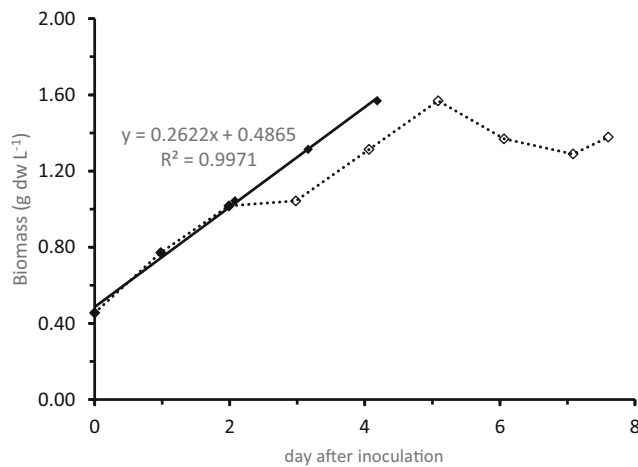


Fig. 8 Biomass growth of *Chlorococcum littorale* (2) in the 6 m³ reactor from 21 November 2011 to 09 December 2011. Productivity results from this period are summarized in Table 5. Note: the 3 top points through which the regression line is drawn, were displaced 0.9 days back (to the left) from their actual position as a consequence of a delay, caused by a foaming incidence that shades the culture in the tubes

Circulation power

Figure 10 shows the volumetric power consumption of the circulation pump as a function of flow velocity. The recirculation energy applied for the duration of the batch was not directly measured, but for this and two following batches, a total of 1107 kWh was used by the circulation pump in the 6 m³ photobioreactor. Divided by the number of operating hours during the period and volume of the system, an average volumetric power supply of 166 W m⁻³ resulted.

Fig. 9 Lipid fraction of biomass, TFA fraction of biomass, predicted TFA, specific irradiation and nitrate concentration under nitrogen limited *Neochloris* in the 6 m³ tubular reactor from 22 October 2011 to 07 November 2011. TFA was predicted according to the inverse nitrogen quota method

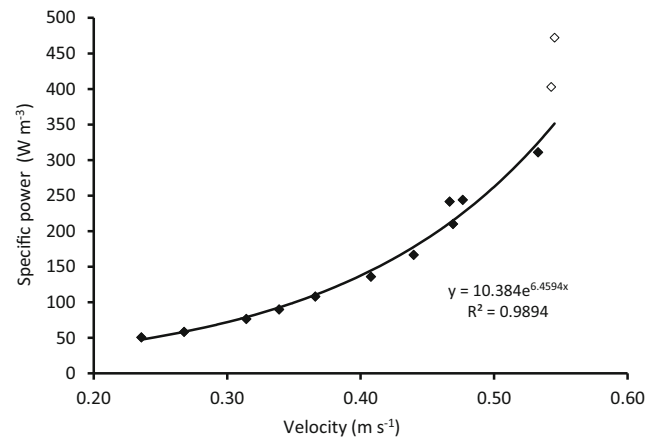
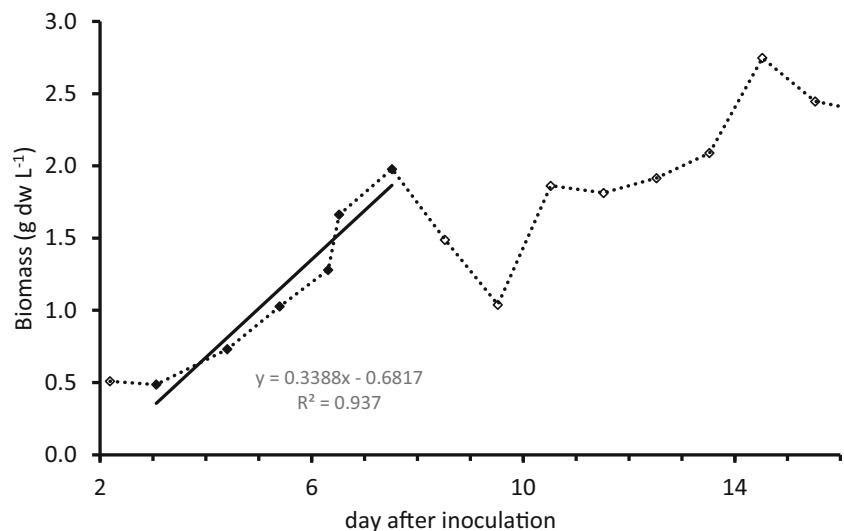


Fig. 10 Volumetric power consumption versus flow velocity in the 6 m³ tubular reactor on growth medium. Note: the points with open signature are not included in the regression

Discussion

Biomass productivity

Neochloris oleoabundans biomass productivity obtained in the soft sleeve tubular photo bioreactor was not very high; 0.11 g dw L⁻¹ day⁻¹ as opposed to that of *C. littorale*, (0.26/0.34 g dw L⁻¹ day⁻¹ under roughly the same irradiation conditions (Table 4). Photosynthetic efficiencies were 1.4, 4.4, and 4.7% respectively. These are winter values, obtained at irradiation levels around 10 MJ m⁻² day⁻¹. When comparing with values obtained in other systems, it should be kept in mind that the current areal productivity and photosynthetic efficiency re-

sults are based on the projected area of the tubes, rather than the full footprint of the photostage. The tubes in the current system on the other hand, are lying directly on the ground while tubes in other horizontal tubular reactors may be separated and lifted over a reflective substrate, resulting in irradiation of the lower part of the tubes by reflected light, for example (Fernandez et al. 2001; Cabanelas et al. 2017) which complicates comparisons. Cabanelas et al. (2017) obtained a nutrient replete, volumetric productivity of $0.64 \text{ g dw L}^{-1} \text{ day}^{-1}$ of *C. littorale* in a horizontal tubular photobioreactor under Dutch summer conditions with high irradiation ($30.1 \text{ mol photons m}^{-2} \text{ day}^{-1}$, corresponding to $15 \text{ MJ m}^{-2} \text{ day}^{-1}$). A photosynthetic efficiency average of 1.5% was obtained with *Nannochloropsis* sp. in a horizontal tubular reactor in the Netherlands during July and August, whereas a vertically stacked tubular photobioreactor yielded 2.4% on average (Vree et al. 2015).

Minimum inoculum biomass concentration for *Neochloris oleoabundans*

A minimum of inoculum concentration of 0.3 kg m^{-3} had been predicted necessary for the applying irradiation. Biomass growth (Fig. 6) appeared to be approximately linear over the first 10 days of the batch cycle with no lag phase or other signs of photoinhibition in the beginning, even though the biomass concentration at inoculation was only 0.14 kg m^{-3} . It is possible that the inoculum had been more properly adapted to the outdoor conditions and therefore could sustain a higher irradiation or stronger dilution than the laboratory culture used to derive the minimum inoculum density. Two other circumstances in terms of irradiation distinguish the 6 m^3 reactor from the laboratory reactor used to derive the minimum inoculum biomass concentration: it includes a large dark volume (2.5 m^3) in manifold and degasser and buffer tanks; this potentially could have allowed the biomass to recover from an irradiance that could otherwise have resulted in photodamage. It is thus difficult to conclude that a biomass concentration of 0.141 kg m^{-3} in general is sufficient to set up a 6 cm diameter tubular reactor system at an irradiation of $13 \text{ MJ m}^{-2} \text{ day}^{-1}$. Further fluorescence work in the laboratory could elucidate that.

Inverse nitrogen quota

The accuracy of the inverse nitrogen quota method to predict the microalgal TFA content is not yet clear—Fig. 9 shows a discrepancy between the predicted and the realized fatty acid concentration. The discrepancy may indicate a difference between the continuous light conditions under which Eq. 6 is derived and the natural day-light cycle under which the outdoor cultivation

is done. Predicted and actual TFA content converge, on the other hand, towards the end of the campaign. This might suggest that the discrepancy is connected to the strong drop in specific irradiation between day 9 and 13 (Fig. 9).

Janssen et al. (2018) found a maximum TAG yield on light between 11 and $26 \mu\text{mol photons s}^{-1} \text{ g dw}^{-1}$. Average (24 h) biomass specific irradiation for *N. oleoabundans* appears to be correlated with TFA and lipid concentration after NO_3 is depleted (Fig. 9). TFA appears to increase at a biomass specific irradiation above $4 \mu\text{mol}_{\text{PAR}} \text{ photons s}^{-1} \text{ g}^{-1}$. Having continued the batch till it had reached a higher biomass concentration would have been the general obvious action to reach a higher fatty acid content and inverse nitrogen quota—if weather conditions had allowed it. Another way to raise the TFA content could have been to reduce the biomass density in the reactor as the biomass specific irradiation appears to be critical to obtain a high TFA yield.

The inverse nitrogen quota is thus potentially a useful tool to help steer the fatty acid content in the biomass.

Circulation power

Specific circulation power was 166 W m^{-3} and per kilogram biomass produced, 28.7 kWh was used. For a tubular plant of this design, we had estimated that a circulation power requirement of $76\text{--}133 \text{ W m}^{-3}$ at a flow velocity of 0.5 m s^{-1} , depending on tube hydraulic roughness. (Norsker et al. 2012). With seawater medium in the current plant, the volumetric power consumption as a function of tube flow velocity is shown in Fig. 10. The regression line equation was $y = 10.384 e^{6.4594 x}$, y being volumetric power consumption (W m^{-3}) with x being tube flow velocity (m s^{-1}). A flow velocity of 0.5 m s^{-1} would thus require 262 W m^{-3} , about double as high as the calculated value. There can be several reasons the actual specific circulation power requirement is higher than calculated, including higher hydraulic friction in the manifold system than anticipated, trapped gas bubbles etc. In addition to optimizing the hydraulic performance of the system, a velocity reduction is an effective way to reduce pumping costs.

General performance

The soft sleeve horizontal tubular reactor described here was constructed at relatively low costs. Strategies for culture propagation, inoculation of outdoor reactors, pH, and oxygen control worked well during the cultivation period.

The horizontal soft-sleeve tubes used were generally convenient for the microalgal production with a few critical points: the horizontal tubes accumulate gas pockets on the high spots if the ground is not sufficiently leveled. Foam or

gas pockets must be removed to maintain light absorption and flow rate. This can in principle be achieved by increasing the circulation pump but there is a limit to how much pressure the tubing can withstand (burst pressure of the used polyethylene sleeves was 0.8 bar, but a 0.25 bar stand-pipe was included to prevent tube bursting and this effectively prevented proper evacuation of tubing using the circulation pump. Instead, a roller device was used to drive air-pockets out. Secondly, emptying the plant for harvest is laborious and discarding the tubing is a sustainability issue.

Author contributions Niels-Henrik Norsker Laboratory and field work. Preparation of manuscript.

Maria Cuaresma Laboratory work and data preparation, review of manuscript.

Jeroen de Vree Laboratory and field work.

Maria Carmen Ruiz-Domínguez Laboratory work.

Maria Carmen Marquez García Laboratory work.

Pauliina Uronen Overall study planning, review of manuscript.

Maria Barbosa Review of manuscript.

René Wijffels Project coordination, review of manuscript.

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Declarations

Conflict of interest Pauliina Uronen is an employee of Neste Corporation. The other authors declare that they have no conflict of interest.

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