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Valorization of *Chrysanthemum* juice as cultivation medium for the microalga *Tetradesmus obliquus*: a proof-of-concept study at pilot-scale

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ABSTRACT

To make the production of certain feedstocks more sustainable, a more circular economy is warranted. One such example is the approach in which green waste biomasses are processed by separating the solid (fiber) fraction from the liquid (juice) fraction to use both fractions as feedstocks for different applications. The liquid fraction can potentially be used as an alternative for mineral medium for algal cultivation. However, little research has so far been done on the implementation of such approach in the production process of microalgae. To provide more insights in this, the potential of flower juice as an alternative for mineral medium was tested to grow microalgae with an emphasis on the potential of using the algal biomass as feed. For this *Tetradesmus obliquus* was cultivated in the juice of *Chrysanthemum*, the most cultivated ornamental plant in the Netherlands. Both laboratory and pilot-scale cultivation showed the potential of the *Chrysanthemum* juice as a replacement for mineral medium. The algal biomass composition in the pilot-scale test showed to be similar to previously reported studies with a protein content up to 58% and a lipid content up to 18% based on dry weight. While the total obtained dry weight, in our study, was lower in the *Chrysanthemum* juice based medium (0.4 g/L) compared to the mineral medium (0.8 g/L), the extended pilot-scale cultivation showed adaptation of the algae to the *Chrysanthemum* juice indicating the potential of using this flower juice as a more sustainable alternative for mineral medium. However, more research into the microbial load and presence of pesticides in the algal biomass after growth on the *Chrysanthemum* juice based medium should be performed.

1. Introduction

Microalgae have received a lot of attention in recent years due to their potential for several applications [1,2]. Using sunlight and CO_2 they can produce biomass rich in proteins, lipids and polysaccharides, forming valuable feedstock for multiple sectors [1–3]. Next to this, several species are able to grow in side- or waste streams [4,5]. As such, one can shift from a more linear to a more circular economic model in which side- or waste streams are used rather than mineral medium [6–8]. However, while microalgae have long been studied, the practical implementation at larger scale still lags behind. Several factors play a role in this, such as high cultivation costs, limited number of algal species investigated, legislative hurdles and the majority of research done at laboratory-scale contribute to the difficult implementation of large-scale microalgae cultivation [4,9–11]. Furthermore, the current large-scale cultivation of microalgae still relies on large amounts of mineral medium (nutrients) and (fresh)water, increasing the environmental footprint and cost of algal cultivation [9,11,12]. To increase the sustainability of algal cultivation, many studies, often limited to laboratory-scale, focus on the use of urban or industrial (municipal) wastewaters as an alternative for mineral medium [4,5,11] or more recently, digestate from anaerobic digesters [13,14]. However, green waste biomasses can form an interesting alternative. When the fiber and liquid fraction (green juice) of the biomass are separated one can use both fractions in different applications. The fiber fraction can be used for feed and insulation while the green juice can be used for the production of microalgae [9,15,16]. To evaluate the potential of cultivating microalgae using a green juice from residual plant material, the potential of flower juice from *Chrysanthemum* waste material was examined as an alternative to mineral medium for algal cultivation. *Chrysanthemum* is one of the most important ornamental plants. In the European Union (EU), the Netherlands are one of the leading countries regarding

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Chrysanthemum cultivation. In 2024, *Chrysanthemum* cultivation in the Netherlands covered approximately 490 ha, primarily in open-field greenhouses [17,18]. Harvesting occurs weekly throughout the year, generating a continuous stream of residual biomass. While the exact volume of this waste stream is unknown, it is estimated to be around 10 kilotons per year based on data from other studies [19]. As such the juice from *Chrysanthemum* flowers could provide an interesting alternative for mineral medium for algal cultivation. Algae are currently regarded as an interesting feedstock in the EU, particularly also to combine algae cultivation with the use of waste streams [20].

Presently only a limited number of studies are available on the use of green juice to cultivate microalgae and they are mostly limited to laboratory-scale tests, often on frozen and/or sterilized green juices [9,21,22]. To provide more insights on the larger scale potential of using non-frozen and non-sterilized green juices, stored for a prolonged period, as a replacement for mineral medium, a proof-of-concept study was conducted. For this we cultivated *Tetradesmus obliquus* at pilot-scale and investigated the potential application of the algal biomass as animal feed. *T. obliquus* was used as it is an algal species tolerant to more adverse conditions [23,24] and able to grow on different types of organic streams [8,25].

2. Materials and methods

2.1. Microalgae strains and culture conditions

Tetradesmus (formerly known as *Acutodesmus or Scenedesmus) obliquus* (SAG 276-3d) was purchased from SAG (Department of Experimental Phycology and Culture Collection of Algae, University of Goettingen, Germany). The axenic stock culture was maintained in 250 mL conical flasks on an orbital shaker at 90 rpm in an incubator at 22 °C under a 16/8 h light/dark cycle with 15 μmol/m^2·s light exposure (cool-white fluorescent). For the laboratory experiment and upscaling, 70 μmol/m^2·s light exposure was used. The freshwater medium for the laboratory experiment and upscaling was autoclaved at 121 °C for 20 min and was based on the SAG basal medium version 10.2008. The approximate composition was: 248 mg/L HNO_3, 21 mg/L H_3PO_4, 247 mg/L KOH, 6.3 mg/L Fe-DTPA, 42 pg/L $CuSO_4·5H_2O$, 2.8 μg/L $ZnSO_4$, 7.2 μg/L $MnSO_4$, 4.3 μg/L Na_2MoO_4, 40 μg/L $Na_2B_4O_7$, 0.2 g/L $NaHCO_3$ and 23 mg/L $MgSO_4·7H_2O$. For the laboratory-scale tests, inoculum was produced by cultivating axenic *T. obliquus* stock cultures to an OD_{720} of approximately 1.2 in 1 L aerated (ambient air) bottles. For the pilot-scale tests, 3 bottles with a 10 L axenic culture of *T. obliquus* with a dry weight of approximately 0.8 g/L were used as inoculum.

2.2. Preparation, storage of Chrysanthemum juice and residual pesticides estimation

The *Chrysanthemum* juice was produced using residual biomass from *Chrysanthemum x morifolium* (various cultivars) obtained from Arcadia Chrysanten, a *Chrysanthemum* grower in the Netherlands. The cultivars present in the residual stream varied and were collected weekly as mixed batches in February 2025. The residual biomass consisted of *Chrysanthemum* stem bases, leaves, and non-marketable flowers. This material was shredded and pressed on the same day of collection using a REKO 260 SP screw press (Fig. S1) to separate the solid fraction (fibers) from the liquid fraction (juice) in an approximate 60:40 (w/w) fiber:juice ratio on a fresh weight basis. This ratio is consistent with previous measurements of residual *Chrysanthemum* biomass. The obtained green (flower) juice from pressing the *Chrysanthemum* flower residual material, hereafter referred to as *Chrysanthemum* juice, was subsequently transferred to a fermentation vessel, where it underwent spontaneous anaerobic fermentation (due to native flora) during a week at ambient temperature. During this period, larger particles settled naturally at the bottom of the vessel, resulting in a clarified upper fraction. Approximately 90% of the total vessel volume formed this clarified fraction, which was carefully drawn from above the sediment layer to obtain clear juice. This process was repeated several times over the span of a month, and the resulting batches were combined into an intermediate bulk container (IBC) to obtain a sufficient volume for subsequent steps. Furthermore, pooling these batches leads to a more homogenous juice. From such fermented *Chrysanthemum* juice, two batches were subsequently used for the cultivation experiment. Batch 1 consisted of 20 L of juice stored indoors, in two plastic 10-L containers, for 3 months at room temperature prior to use in the laboratory-scale test. The nutrient content was analyzed prior to the laboratory-scale test. Batch 2 consisted of approximately 400 L of *Chrysanthemum* juice in a black 600-L IBC with an opening at the top, semi-closed by a lid that allowed overpressure to leave the barrel. This container was subsequently stored outside at ambient temperatures for a long-term storage test from the 21st of February 2025 to the 23th of June 2025 (12 L batches of juice were taken from the container at the start and during the growth experiments). Throughout the storage of the juice, samples were manually taken from the top of the container, by opening the lid, to analyze, in triplicate, the evolution of the total Kjeldahl-N (TKN), PO_4^{3-}, NO_3^- (converted to NO_3^--N, see below), and NH_4^+-N present in the juice. The pH was also monitored during the storage. By performing a long-term storage experiment the stability of the aforementioned components could be tested to evaluate whether a steady state equilibrium could be reached after a certain storage period during which spontaneous fermentation occurred. The *Chrysanthemum* juice was stored in a low-tech way representative of how farmers would store it. It was not treated by any sterilization or filtration prior to being used for the cultivation, as a proof of concept of using minimally treated, spontaneously fermented juice and to evaluate the evolution of the tested nutrients. This was done to reduce the costs of extra steps and to minimize potential alterations to the juice chemistry due to e.g., autoclaving [11,26,27].

Given that pesticides are used in the cultivation of ornamental flowers, the presence of residual pesticides in the juice after pressing is possible. To determine whether the fermented *Chrysanthemum* juice contained any residual pesticides, the samples, taken after 52 days of fermentation, were screened against a comprehensive database of 450 pesticides, including insecticides, fungicides and herbicides. The pesticides residue analyses were conducted using a gas chromatography-triple quadrupole mass spectrometry (GC–MS/MS) and liquid chromatography-triply quadrupole mass spectrometry (LC-MS/MS) technique. The limit of quantification was set at 0.01 mg/kg. The analysis was performed by Eurofins Agro.

2.3. Microalgal cultivation on Chrysanthemum juice

2.3.1. Laboratory-scale cultivation of T. obliquus on Chrysanthemum juice

To assess the potential of microalgal growth using *Chrysanthemum* juice as the sole nutrient source a laboratory-scale experiment lasting 16 days was run in aerated 1 L bottles, with both the aeration in- and outlet provided with filters (0.2 μm). A total start volume of 1 L was achieved by using a 50 mL algal start culture, equaling approximately a total of 4.11E05 to 5.42E05 cells per mL per bottle. As the nutrient source a total of 41 mL of unfiltered and unsterilized fermented *Chrysanthemum* juice from batch 1 was added with 909 mL of tap water. The 41 mL was based on the NH_4^+-N content of the *Chrysanthemum* juice to match the NO_3^--N content of mineral medium. Sterile freshwater mineral medium (see Section 2.1) was used as a control medium. No pH adjustment was done at the start or throughout the experiment. During the cultivation only ambient air was provided to the bottles. A 50 mL sample for nutrient analysis was taken at the start of the cultivation, at days 7, 12 and at day 14. The dry weight (DW), absorbance at 720 nm (OD_{720}), cell count and pH were measured three times per week (Monday, Wednesday and Friday). Both the *Chrysanthemum* juice condition and the positive control were tested in triplicate.

2.3.2. Pilot-scale cultivation of T. obliquus on Chrysanthemum juice

For the pilot-scale cultivation a Green Wall Panel-II.N.9 photobioreactor (GWP-II.N.9®; Fotosintetica & Microbiologica S.r.l.) was used [28]. The reactor consisted out of 3 single panels (3.4 m in length) made from low-density polyethylene, connected to each other, each panel with a volume of approximately 110 L. The total working volume during the pilot-scale experiments was approximately 340 L (330 L working volume in the bags and approximately 10 L in the tubing, connections between the bags) (Fig. 1).

The 340 L of cultivation volume was composed of a total of 30 L start culture *T. obliquus* (see also Section 2.1), 12 L of the *Chrysanthemum* juice from batch 2 and approximately 298 L of tap water. The juice was manually taken from the top opening of the container, using 2 L bottles. The 12 L was based on the NH_4^+-N content in the juice to match the NO_3^--N content in mineral medium. Both the start culture and *Chrysanthemum* juice were manually added to the algal culture in the pilot-scale photobioreactor through one of the air outlets (see also below). The culture was pumped through the reactor by a centrifugal pump (CO350/03/A; Lowara). Each panel was provided with air flow, for mixing, through an air sparger for bubbling (12 mm perforated O·D RILSAN® tubing) placed at the bottom of each panel and filtered (0.2 μm) ambient air was pumped in using one central air pump (JDK 120; Secoh). The air flow was regulated by air valves that were adjusted manually to provide adequate air flow to each panel. Each panel had 2 air outlets to remove excessive air (pressure). One panel, the middle, was provided with CO_2. CO_2 was injected between 0.5 and 0.9 L/min based on the pH set point through a solenoid valve. A mass flow meter (D-6300/FS; Bronkhorst) was used for monitoring of the CO_2 airflow. The pH set point at which CO_2 was injected was 8.80 and CO_2 was injected until the pH dropped to 8.60. The pH was monitored by a pH 7687 probe (B&C). The temperature of the culture was monitored by a PT100 probe. Both the temperature and pH were logged every minute. The reactor was provided with N.12 LED lamps (MOD. TL PLUS). Each panel was provided with 2 LED lamps that were continuously on during the cultivation. The lamps covered 50% of the surface of each panel and provided 340 μmol/m^2·s PPFD. The reactor was placed indoor in a closed building with little to no natural sunlight. Only 0–10 μmol/m^2·s PPFD of natural sunlight reached the cultivation true small windows in the gates of the building.

The cultivation experiment with *Chrysanthemum* juice ran for a total of 23 days, from the 4th of June to the 27th of June 2025. Throughout this cultivation period, three partial harvests of 200 L each plus one final (340 L) harvest took place. The harvested biomass was freeze dried and stored for further analyses. After each harvest 200 L new tap water was added with 12 L of *Chrysanthemum* juice from batch 2. The biomass was harvested by a two-step centrifugation (RA-20-VX; Rousselet) approach. First, a concentration step was performed by decantation. In this step, the harvested volume of 200 or 340 L was reduced to approximately 2.7 L. Subsequently, the algae were collected by centrifugation using a filter bag (pore size 10–15 μm). The first two harvests took place after a 7-day cultivation (periods 1 and 2), the third after 5 days of cultivation (period

Table 1
Overview of the pilot-scale cultivation experiment for both the Chrysanthemum juice and mineral medium (control) experiments. For the Chrysanthemum juice test, the total cultivation period stretched 23 days, divided in 4 periods of which 3 with a partial (200 L) batch harvest and 1 period (final period, 4) where the total reactor (340 L) was harvested. For the positive control in mineral medium a 7-day test was performed.

Period	Dates	Harvest	Days of cultivation
***Chrysanthemum* juice based medium**			
1	4–11 June	200 L	7
2	11–18 June	200 L	7
3	18–23 June	200 L	5
4	23–27 June	340 L	4
Mineral medium (positive control) condition			
5	7–14 July	340 L	7

Fig. 1. The GWP-II.N.9® reactor consisting of three 110 L panels.

3) and the final, total harvest, took place after 4 days (period 4). Table 1 schematically shows the cultivation periods.

No filtration of the tap water was performed for both conditions. The pH set-point and the decision to harvest after 7 days for the two first harvests were based on a preliminary pilot-scale experiment (data not shown) while the harvest at respectively 5 and 4 days for periods 3 and 4 were based on the growth observations during the cultivation. In order to compare the growth in the *Chrysanthemum* juice with standard (mineral) medium, a positive control (period 5) experiment in the same reactor was performed with mineral medium and fresh inoculum from the 7th to the 14th of July 2025 using the same pH set-point.

2.4. Analytical methods

2.4.1. Growth determination

For the laboratory-scale experiment, the microalgal growth was monitored by cell counting, dry weight (DW) measurements and absorbance measurements at 720 nm (OD_{720}). For the pilot-scale experiment, the growth was monitored daily by cell counting and dry weight measurements, both performed in duplicate. For the pilot-scale experiments, the growth rate (μ) was calculated based on the cell count using the following equation: $\mu = \frac{LN\left(\frac{N2}{N1}\right)}{T2-T1}$ with LN being the natural logarithm, N1 and N2 the cell count measured at the initial (T1) and final (T2) time of the exponential growth phase. A Bürker counting chamber and microscope (DM50, Leica) were used for cell counting. For DW measurements a 5 mL sample was filtered on a pre-weighted glass microfiber membrane (1.2 μm, washed in deionized water). Samples were subsequently dried at 70 °C for 24 h and transferred to a desiccator before weighing. The absorbance at 720 nm was measured with a UV-3100PC Spectrophotometer (VWR). For the laboratory experiment a pH meter (Edge meter, HI11310 probe, Hanna Instruments) was used to determine the pH. For the pilot-scale cultivation the pH (and temperature) was continuously logged by the sensors installed in the photobioreactor (see also Section 2.3.2).

2.4.2. Nutrient content of Chrysanthemum juice and uptake during growth

The nutrient content (Kjeldahl-N, NO_3^-, NH_4^+-N and PO_4^{3-}) of the *Chrysanthemum* juice was analyzed throughout the storage and at the start of the cultivation experiments. During the algal growth, both for the laboratory and pilot-scale experiments, the nutrient (nitrogen) uptake was evaluated by measuring total Kjeldahl-N, NO_3^- and NH_4^+-N concentrations. For better comparison with the NH_4^+-N concentrations, the measured NO_3^- content was converted to NO_3^--N, and reported throughout this manuscript as such, using the 0.2258 conversion factor based on the molar masses of N and NO_3^-.

For the laboratory experiment, one 50 mL sample per bottle was taken while for the pilot-scale experiment three samples of 50 mL from the photobioreactor were taken for analyses in triplicate. Samples were centrifuged for 10 min at 1550 ×*g* at 20 °C (Sorvall lynx 4000, Thermo Fisher Scientific) before nutrient analysis. The total Kjeldahl-N was measured using the Kjeldahl method (Gerhardt, Kjeldatherm, Vapodest 20 s). Ion chromatography (Metrohm Eco IC using a Metrosep A Supp 17 – 250/4.0 column) using a 6 mM Na_2CO_3 buffer after filtering through 0.45 μm and 0.20 μm disposable PET-filters (NBN EN ISO 10304-1) was used for NO_3^- and PO_4^{3-} contents determination. For the NH_4^+-N content determination steam distillation (Vapodest 200, Gerhardt) was used. Each sample was diluted in a phosphate buffer and ammonia was expulsed from this weak alkaline solution by distillation (4 min, 100% steam power). Ammonia was subsequently collected in a 2% boric acid solution and volumetrically determined by titration with 0.01 M HCl (CMA/2/I/E.3 ISO 5664:1984).

2.4.3. Quality and safety of the cultivated algal biomass

The harvested biomass was analyzed for microbial (pathogenic) indicator organisms, presence of the heavy metals Pb, Cr and Cd and for nutritional quality. The tested microbial indicator organisms were Enterobacteriaceae at 37 °C (AFNOR BRD 7/24-11/13); beta glucuronidase positive *E. coli* (ISO 16649-2); coagulase positive staphylococci (ISO 6888-1); fungi (ISO 21527-1); yeast (ISO 21527-1) and the total aerobic cell count (30 °C) (ISO 4833-1). For a limited number of samples also the presence of Salmonella (AFNOR BRD-7/11-12/05) and *Clostridia perfringens* (ISO 7937) was analyzed. To assess the microbial safety of the algal biomass cultivated in the *Chrysanthemum* juice, samples were taken from the reactor at the start and end of each period and from the harvested algal biomass itself to screen for microbial presence.

The presence of heavy metals in the freeze-dried algal samples was assessed by analyzing Cd, Cr and Pb presence in the harvested algal biomass from periods 1, 3, 4 and 5. This was done by oven-ASS. Samples were first destructed using a CEM Mars Xpress microwave. For this, 0.20 g of algal biomass was weighed to which 2.5 mL HNO_3 and 7.5 mL HCl was added. Samples were destructed at 190 °C for 25 min. Afterwards the samples were placed in a 100 mL plastic recipient and diluted until a final volume of 100 mL. Samples were analyzed with an oven-ASS (Thermo Fisher Scientific ICE 3000 series AA spectrometer). Measurements were performed in triplicate.

The nutritional quality of the biomass was analyzed through a Weende analysis. The Weende analyses consisted out of analyzing the moisture content (ISO 1442), raw fat content (ISO 1443), inorganic (ash) substance (mineral fraction, ISO 936), crude fiber content (SM00121), starch content (Ewers method SM00120) and crude protein level (N-containing substance × 6.25 or × 4.78 following ISO 1871) of the algal biomass. The lower 4.78 conversion factor was also used as the traditionally used 6.25 factor often leads to an overestimation of the protein content in microalgal biomass [29–31]. The Weende and microbial analyses were done by LOVAP NV (Geel, Belgium) following accredited methods.

2.5. Statistical analysis

To compare the differences in cell count, pH evolution, DW and absorbance (OD_{720}) between the mineral medium (control) and *Chrysanthemum* juice based medium at lab scale, a one-way analysis of variance (ANOVA) with post hoc Tukey HSD with a significance level of 0.05 was performed. Normality was tested with a Shapiro–Wilk test. Data are given as mean ± SD. To compare the growth, based on the cell count, between the different periods and conditions for the pilot-scale test a one-way analysis of variance (ANOVA) with post hoc Tukey HSD with a significance level of 0.05 was performed.

3. Results and discussion

3.1. Long term storage of Chrysanthemum juice for pilot-scale growth experiments and presence of pesticides

Residual biomass from *Chrysanthemum* flowers is readily available and rich in nutrients, however, currently it is mostly considered as a waste material and discarded [32]. Yet, when the fiber fraction is separated from the liquid fraction, this fraction could be used as a nutrient source for algae cultivation. To test the long-term storage and stability of the juice regarding the nitrogen content (NH_4^+-N, NO_3^--N and Kjeldahl-N), PO_4^{3-} content and pH, a long-term storage of the *Chrysanthemum* juice was tested by storing the juice at ambient outdoor conditions for up to 17 weeks (122 days). The temperature varied significantly during the storage period, from winter to summer, ranging from 1.5 °C to 34.3 °C with an average temperature of 13.6 °C (Fig. S2). Fig. 2 shows the evolution of the pH, nitrogen (NO_3^--N; NH_4^+-N and Kjeldahl-N) and phosphorus (PO_4^{3-}) contents during this storage period.

While the juice had a high initial NO_3^--N content of 1459 mg/L, it was completely removed after approximately 80 to 90 days. Contrary, the $NH4^+$-N content steadily increased from the start (250 mg/L) until day

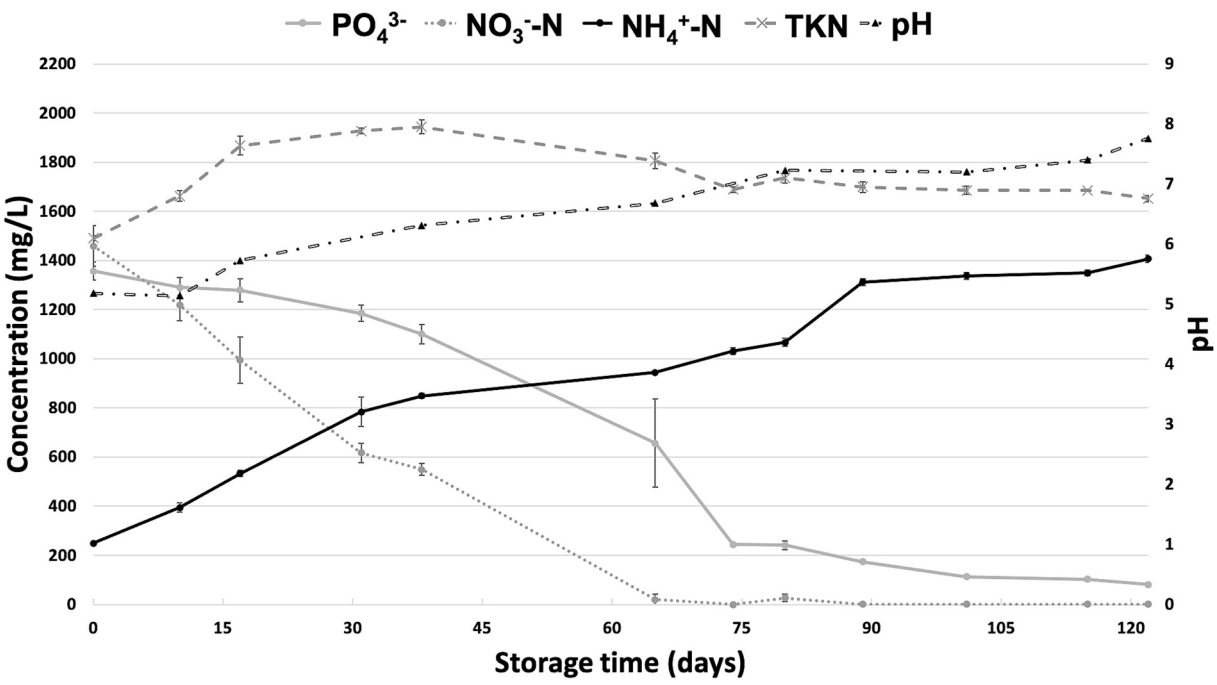

Fig. 2. Evolution in mg/L for NO_3^--N; NH_4^+-N, total Kjeldahl-N and PO_4^{3-} content (left Y-axis) and pH (right Y-axis) over time during the 122 days storage of the Chrysanthemum juice. Measurements were performed in triplicate (excl. the pH measurements) and the error bars show the SD values.

89 where it leveled off and remained stable until day 122 between approximately 1300 and 1400 mg/L (Fig. 2 & Table 2).

This change in composition could be explained by the process of (fermentative) dissimilatory nitrate reduction to ammonium (DNRA), a process where nitrate, through nitrite, is reduced to ammonium [33,34]. Since the $NH4^+$-N content remained relatively stable from day 90 till day 122, it was possible to always use the same volume (12*L*) of *Chrysanthemum* juice to be added to the photobioreactor as a nitrogen source. A stable waste stream, especially in terms of nutrient composition, would be highly desired as waste streams often differ in nutrient composition [35,36]. The total Kjeldahl nitrogen content fluctuated throughout the storage between 1490 and 1944 mg/L. During the storage it is possible that also nitrogen gas (N_2) was formed and lost [34,37], however, this was not monitored. During storage, the measured PO_4^{3-} content was slowly reduced from 1357 to 81 mg/L after 122 days.

Table 2
Comparison between the composition of Chrysanthemum juice and mineral medium. For reference, the composition of the tap water used is also shown (mg/L). The number between brackets in the Chrysanthemum juice column gives the theoretical concentration when 12 L Chrysanthemum juice, stored for 101 days, is added to 298 L of tap water (without taking the composition of tap water into account). Composition for the Chrysanthemum juice and tap water was determined by an external analytic laboratory, except for NO_3^--N, NH_4^+-N and PO_4^{3-}. The composition of the mineral medium was calculated based on the recipe of the mineral medium without taking the composition of tap water into account.

Composition (mg/L)			
	Chrysanthemum juice	Mineral medium	Tap water
NO_3^--N	0.70 (0.03)	56	0.45
NH_4^+-N	1337.19 (53.49)	0	<0.05
PO_4^{3-}	113.39 (4.54)	21	<10
K	6685.76 (276.43)	172	6.1
S	141.09 (5.64)	3	18.4
Fe	2.54 (0.10)	0.75	0.007
Cu	0.07 (0.0028)	1E-08	0.00005
Zn	2.06 (0.08)	0.001	0.002
Mn	1.15 (0.046)	0.003	0.00005
Na	66.67 (2.67)	0.06	14.5
Mg	330.55 (13.22)	2.24	7

This can be explained by the biological activity of the microorganisms in the juice using the PO_4^{3-} as a phosphorus source [38]. The phosphorus as such is removed from the juice and incorporated by the microorganisms growing in the juice. In general, the *Chrysanthemum* juice (Table 2) contained, after 101 days of storage (start of period 1), a low amount of phosphorus, 36.98 mg/L (based on the 113 mg/L PO_4^{3-} content) compared to the N content (1337 mg/L NH_4^+-N), giving a 36:1 ratio, deviating from the commonly applied Redfield ratio of 16:1 (N:P). This indicates the *Chrysanthemum* juice is P-limited [39]. However, as previously mentioned, the phosphorus is most likely incorporated in the biomass of the micro-organisms present in the juice, meaning that an extra filtration step to sterilize the juice would also remove the phosphorus present in the micro-organisms that otherwise potentially could be used by the microalgae. Furthermore, studies have already demonstrated the benefit of algae-bacteria consortia [36,40]. The pH of the *Chrysanthemum* juice slowly increased from 5.18 (day 0) to 7.76 (day 122) during storage. However, to elucidate the complete mechanisms of the evolution of the different nitrogen and phosphorus species (over a longer time) and exact microbiological processes, more in-depth experiments will be needed. Many studies investigating the potential of separating the solid from the liquid fraction focus mostly on the solid fraction and have less attention to the liquid fraction and the (long-term) storage or stability of the juice [41,42]. The studies that did look into juices from green waste materials mainly studied the extraction of valuable components from the juice or the potential of the juices for biogas (energy) production. These studies had little attention to the (long-term) storage influence on the nitrogen and phosphorus content in the juice or focused on other parameters during storage [43–45]. The limited studies available using green juices for other purposes such as microbial growth however often used fresh, frozen or short term stored juice in small volumes making comparison with the results described here difficult [9,21,22,46].

From the insecticides tested only flonicamid (0.42 mg/kg) and sulfoxaflor (0.05 mg/kg) were detected in the fermented *Chrysanthemum* juice. However, these residual presences of flonicamid and sulfoxaflor are higher than the maximum residue level in algae products (0.03 mg/kg for flonicamid and 0.01 for sulfoxaflor respectively) allowed in the EU [47]. From the fungicides tested, both boscalid and fluopyram were

detected at 0.01 mg/kg; which was the limit of quantification and also the threshold limit for feed (0.01 mg/kg) in the EU [47]. The fungicide mepanipyrim was detected at 0.09 mg/kg, which is substantially above the maximum residue level of 0.01 mg/kg [47]. One shortcoming of our test is that we only tested the residual presence of these pesticides in the fermented *Chrysanthemum* juice. Further tests on the algal biomass itself would be needed to detect the actual presence in the algal biomass.

3.2. Microalgal growth on Chrysanthemum juice and nutrient content evolution at laboratory-scale

To assess the potential of the *Chrysanthemum* juice for microalgal growth, a laboratory experiment in 1 L bottles was performed. The total volume of *Chrysanthemum* juice, 41 mL, added was based on the NH_4^+-N concentration in the *Chrysanthemum* juice and recalculated to match the NO_3^--N content of the mineral medium (ca. 55 mg/L) to provide approximate equal concentrations of N to the algae, albeit a bit higher compared to the N from the NO_3^- in the mineral medium (Table 3).

After 14 days of growth the NH_4^+-N was depleted in the *Chrysanthemum* medium while the mineral medium still had NO_3^--N present (Table 3), the growth however already slowed down in the latter (Fig. 3). The experiment was therefore halted after 16 days. Fig. 3 depicts the results over the 16-day period.

The cell count ($p = 0.7963$), DW ($p = 0.2293$), absorbance ($p = 0.7136$) and changes in pH ($p = 0.619$) showed no statistical difference over time between the mineral medium (control) and the *Chrysanthemum* juice based medium. However, both the OD and DW measurements were less reliable the first 5 days (Fig. 3) compared to the cell count, due to interference of juice based compounds at low algal concentrations. For this reason, OD measurements were not performed for the pilot-scale tests, DW measurements were however still performed. In general, our results show that *Chrysanthemum* juice can be used to cultivate *T. obliquus* without detrimental effects on its growth compared to the mineral medium. After 16 days the cell count and dry weight were even higher compared to the mineral (control) medium. A maximum average dry weight of 0.66 g/L was obtained for the *Chrysanthemum* juice compared to 0.45 g/L for the mineral medium (Fig. 3b). The obtained dry weights in this study were similar or better to previous studies using *Tetradesmus* species in wastewaters [8,48–50]. Given that the *Chrysanthemum* juice was used without filtration or sterilization, one can argue that the higher DW obtained in the *Chrysanthemum* juice compared to the mineral medium was due to the growth of other organisms as well. However, this effect was mainly seen at the first 2 to 5 days for the DW and OD measurements that show a substantial increase compared to the mineral medium, while this was not reflected in the cell count data of the *Chrysanthemum* juice based medium (Fig. 3A, B and C). This sharp increase in DW and OD the first few days can be attributed to the growth of other organisms and presence of organic material. Interestingly, at the end of the cultivation a higher total cell count was achieved for the *Chrysanthemum* juice based medium, 1.58E07 cell per mL, compared to the mineral medium, 8.81E06 cells per mL, indicating the successful cultivation of *T. obliquus* in the *Chrysanthemum* juice. Furthermore, visual observations during microscopic cell counting seemed to indicate a decrease in microbial presence throughout the cultivation period. The higher cell counts or DW results in the *Chrysanthemum* juice highlights the potential of using more complex waste streams to grow algae. This concurs with other studies that have already highlighted the potential use of wastewaters to cultivate microalgae, often attaining a similar growth in the medium based on wastewater compared to mineral medium [8,11,50,51]. To further explore the potential of the *Chrysanthemum* juice, a pilot-scale test was performed given the difficulties often attributed to the scaling-up process of algal cultivation from laboratory to larger scale cultivation.

Table 3
Nutrient concentrations (mg/L) for NO_3^--N, NH_4^+-N and Kjeldahl-N at the start and after 14 days of the laboratory-scale experiment. The mean and standard deviation are shown ($n = 3$).

		NO_3^--N (mg/L)	NH_4^+-N (mg/L)	Kjeldahl-N (mg/L)
		Mean ± SD	Mean ± SD	Mean ± SD
Mineral medium	Start	52.85 ± 0.30	0.00 ± 0.00	0.00 ± 0.00
	End	20.58 ± 2.14	0.00 ± 0.00	3.22 ± 0.20
Juice based medium	Start	1.64 ± 0.02	67.75 ± 0.29	81.40 ± 3.67
	End	0.00 ± 0.00	0.00 ± 0.00	5.04 ± 4.68

3.3. Microalgal growth on Chrysanthemum juice at pilot-scale

To test the potential of *Chrysanthemum* juice at larger-scale, a pilot-scale experiment was performed in a flat plate panel reactor with a total working volume of 340 L between the 4th and 27th of June 2025 (periods 1–4). To compare the data from the *Chrysanthemum* juice based medium, a positive control experiment in mineral medium was performed between the 7th and 14th of July 2025 (period 5). In total 12 L of the *Chrysanthemum* juice was added as the sole nutrient source for the algae at the start of each period (1–4) to obtain a similar nitrogen content, approximately 55 mg/L, compared to the mineral medium (Table 4).

The *Chrysanthemum* juice generally contained sufficient micronutrients comparable to the mineral medium. However, the PO_4^{3-} content was 5 times lower compared to the mineral medium (Table 2). Fig. 4 shows the measured temperature and pH during the 5 periods the algae were cultivated.

The pH remained stable between the 5 periods, with an average between 8.58 and 8.65. The average temperature however differed approximately 5 °C between period 1 and the other periods (approximately an average temperature of 25 °C in period 1 vs 30 to 31 °C in the other periods). Fig. 5 shows the growth (cell count) of *T. obliquus* in the *Chrysanthemum* juice. For the first two periods a lag phase of 2 days was visible after which the cells entered an exponential phase. For periods 3 and 4 no lag phase was observed after the partial harvest and dilution (black arrows), the cells immediately entered the exponential growth phase (Fig. 5) which was comparable with the absence of a lag phase in period 5, the positive control (Fig. S3b). Furthermore, after only 2 days of growth the stationary phase was already reached (Fig. 5).

Microscopic analysis of the cells also showed severely stressed algae during the first 2–3 days of periods 1 and 2 while from period 3 on, little to no stress was observed. This could be due to the adaptation of the cells to the *Chrysanthemum* juice as microalgae often need an acclimatization period when grown in new or adverse conditions [52–55]. Another potentially contributing factor was the lower start cell concentration in the first two periods compared to periods 3 and 4; this was especially the case for period 1. Different initial cell concentrations can influence the growth [56,57]. The differences in growth patterns between the two first and two last periods could also be observed in the pH profiles of those periods (Fig. 6).

During periods 1 and 2 the pH only slowly started to rise to 8.80 after two days, while for periods 3 and 4 the pH started to rise already on the first day, reaching the set-point approximately after 1 day. Once the set-point was reached, a continuous increase and decrease in the pH could be observed indicating algal growth (Fig. 6). The difference in pH evolution between the juice based medium and the mineral medium can also be observed in the pH profile of the latter where the pH rises faster (from the start on), indicating faster adaption (no lag phase) and growth (Fig. S3a). This could also be seen in the cell counts of the positive control, where no lag phase was observed and higher final cell counts were reached (Fig. S3b). During period 4 a slight increase in cell count was still observed at day 23 compared to day 22, this could potentially be due to more $NH4^+$-N still present (22 vs 10–11 mg/L; Table 4) compared to the other periods. However, taking into account the SD values only a slight difference in cell counts was observed between day

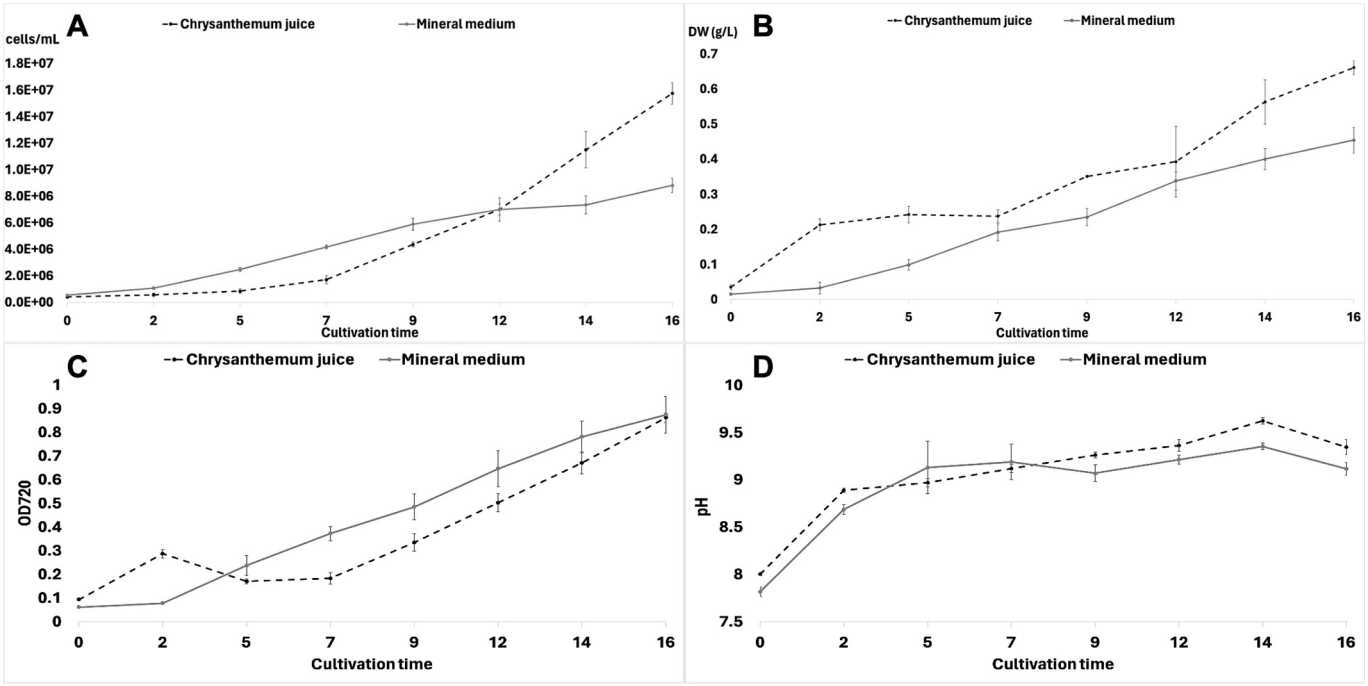

Fig. 3. Growth and pH changes over a 16-day period during the laboratory experiment. A. Cell concentration (cells/mL); B. DW (g/L); C. Absorbance (OD720); and D. pH. Grey solid lines show the growth and pH changed in the mineral medium and the dashed black line shows the results in the Chrysanthemum juice ($n = 3$). Error bars show the standard deviation.

Table 4
Nutrient concentrations for NO_3^--N, NH_4^+-N and Kjeldahl-N at the start and end of each period of the pilot-scale test. Mean concentrations (mg/L) and standard deviation (SD, $n = 3$) are shown. The periods are defined in Table 1 and refer to each growth period between start and (partial) harvest for each cultivation period. Start and end refers respectively to the start and end concentration of the nutrients measured.

Period	Start or end	NO_3^--N (mg/L)	NH_4^+-N (mg/L)	Kjeldahl-N (mg/L)
	Period	Mean ± SD	Mean ± SD	Mean ± SD
		***Chrysanthemum* juice based medium**		
1	Start	5.49 ± 0.02[a]	51.19 ± 1.05	80.51 ± 2.83
	End	1.91 ± 0.01	11.04 ± 0.34	33.67 ± 6.71
2	Start	0.90 ± 0.01	55.40 ± 0.95	56.37 ± 3.79
	End	0.08 ± 0.01	10.70 ± 0.78	26.39 ± 16.63
3	Start	0.40 ± 0.08	53.71 ± 1.09	42.92 ± 4.34
	End	0.05 ± 0.05	10.91 ± 2.37	39.55 ± 15.74
4	Start	0.07 ± 0.07	53.62 ± 0.96	92.09 ± 10.21
	End	0.01 ± 0.01	22.37 ± 4.67	14.20 ± 2.52
		Mineral medium (positive control) condition		
5	Start	54.83 ± 0.08	1.57 ± 1.34	17.09 ± 9.41
	End	0.00 ± 0.00	0.00 ± 0.00	10.55 ± 10.40

[a] A relative high concentration of NO_3^--N was measured due to the inoculum still containing HNO_3^-.

22 and day 23 in period 4, indicating the cells also entered early stationary phase. In general, growth of the algae halted when the $NH4^+$-N concentration reached approximately 10 to 11 mg/L (Fig. 5). The exact reason as to why the growth halted while there was still $NH4^+$-N present is uncertain, however, the low concentration of PO_4^{3-} in the *Chrysanthemum* juice could have caused a premature shortage of phosphorus, leading to a growth arrest of the cells (see also 3.1). When looking at the obtained DW the last two periods of the *Chrysanthemum* juice test obtained a DW of approximately 0.41 g/L, nearly double the DW obtained in the first two periods (Table 5). However, this was still only half of the final DW obtained in the mineral medium (Table 5 & Fig. S4). When comparing the cell counts in the *Chrysanthemum* juice, they were

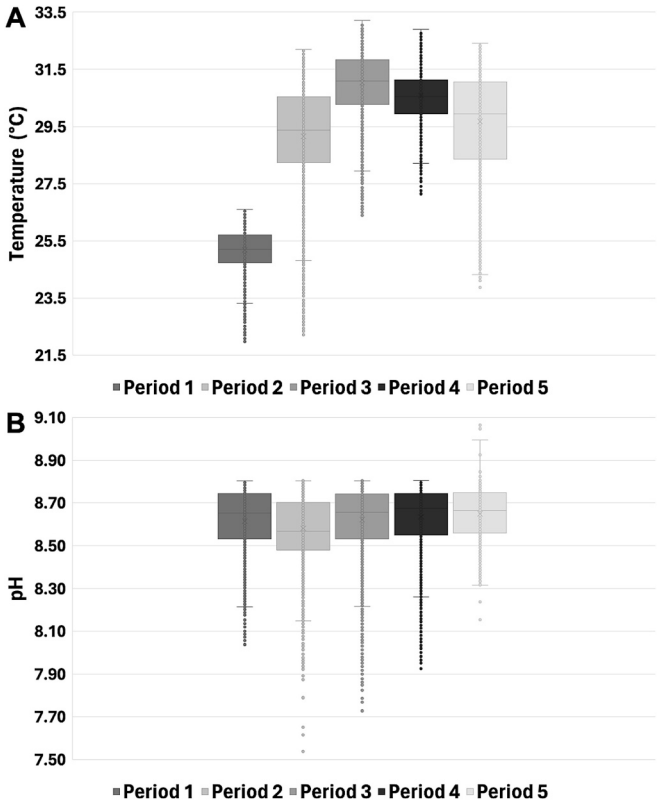

Fig. 4. Box plots showing the temperature in °C (A) and pH (B) measurements during the 5 cultivation periods.

significantly lower than in the mineral medium for all periods ($p < 0.05$). This is confirmed by the DW results (Table 5).

Compared to other studies, the final DW results obtained in the

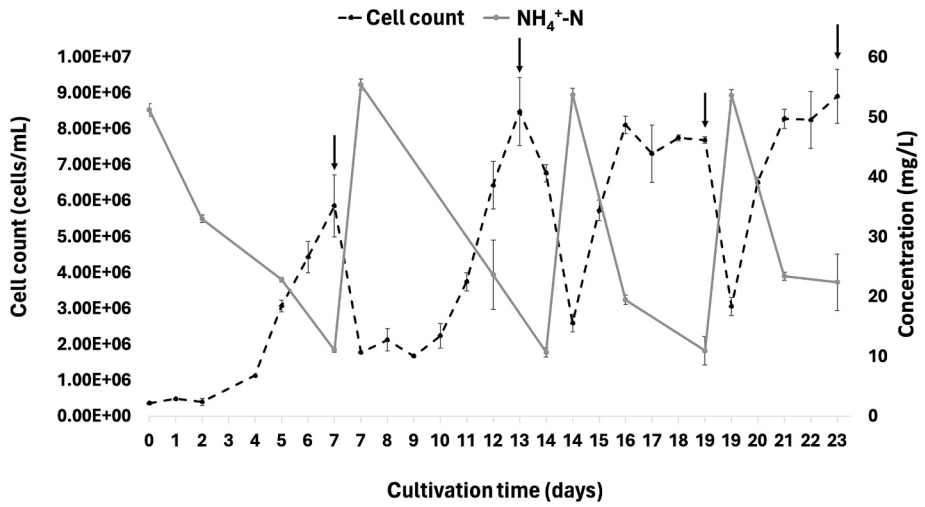

Fig. 5. Cell count (cells/mL; left Y-axis) and concentration of NH_4^+-N (mg/L; right Y-axis) evolution during the cultivation experiment in Chrysanthemum juice. Each harvest is shown by a black arrow. Each measurement was performed in triplicate. Error bars represent the standard deviation.

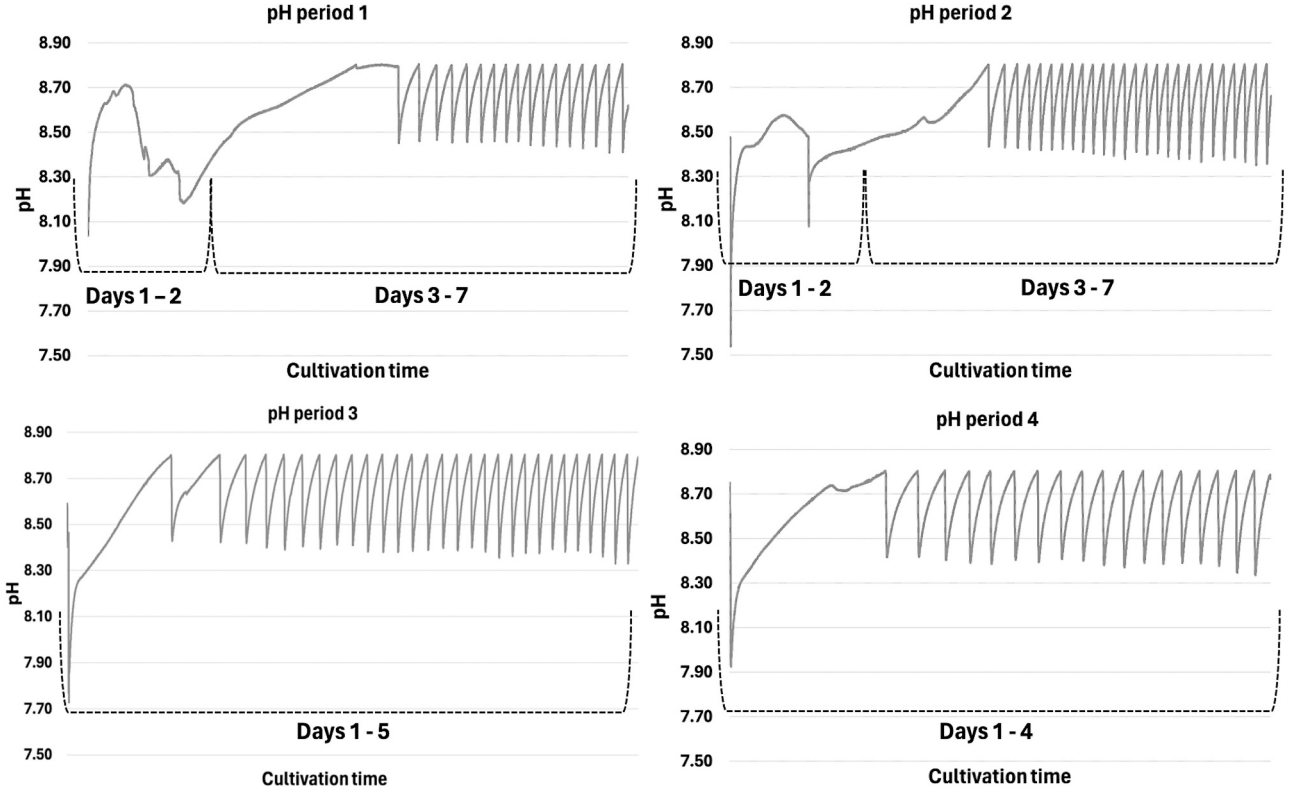

Fig. 6. The pH profiles of the 4 periods during the test with Chrysanthemum juice. The first two periods showed a lag phase of approximately 2 days in which the pH only slowly increased until CO_2 was injected at pH 8.80. Periods 3 and 4 no longer show such a lag phase and the initial rise of the pH is faster.

Chrysanthemum juice is relatively low for cultivation in a photobioreactor. Other studies, that also cultivated *Tetradesmus* sp. In closed photobioreactors, e.g., Malaguti et al. (2024) and Abomohra et al. (2014) reached concentrations up to 3 g/L [58,59], our obtained biomasses (0.4 g/L) are thus significantly lower, but comparable to open pond systems, e.g., Amorim et al. (2021) also reached 0.4 g/L [60]. Yet, even in open ponds, higher concentrations up to 2.5 g/L have been documented [61,62]. However, we performed no optimization, started with a rather low start concentration compared to e.g., Malaguti et al. (2024) and de Marchin et al. (2015) who used a start concentration of 0.4 to 0.5 g/L [58,61]. We also cultivated the algae without any prior acclimatation, solely using *Chrysanthemum* juice as a nutrient source. Furthermore, the *Chrysanthemum* juice was used as such without any filtration or sterilization and in batch cultivation without any medium adjustment prior to cultivation. Further pretreatments, addition of extra *Chrysanthemum* juice during the growth and addition of phosphorus could possibly aid in increased cell counts as could a (semi-)continuous cultivation method. Future studies will be needed to fully explore the potential of the *Chrysanthemum* juice for algal cultivation.

Using *Chrysanthemum* juice might have certain advantages compared to other waste streams. Firstly, using a nutrient source from plant origin makes it possible to obtain an organic certification [63,64]. Especially

Table 5
Growth characteristics of *T. obliquus* in the Chrysanthemum juice and mineral medium during the pilot-scale test. DW = Dry weight. Mean values and standard deviation (SD) are shown ($n = 2$). Period 5 (mineral medium) was significantly different from periods 1 to 4 (Chrysanthemum based medium) compared on cell count and DW.

Period	#Days	Lag phase (days)	Cell count start exp. phase + SD (cells/mL; E+05)	Cell count end exp. phase + SD (cells/mL; E+05)	Growth rate + SD (1/d)	Final DW + SD (mg/L)
***Chrysanthemum* juice based medium**						
1	7	2	3.98 ± 0.99 (Day 3)	58.59 ± 8.62 (Day 7)	0.54 ± 0.02	0.23 ± 0.02
2	7	2	16.72 ± 0.22 (Day 9)	84.84 ± 9.40 (Day 13)	0.41 ± 0.03	0.28 ± 0.02
3	5	0	25.95 ± 2.43 (Day 14)	81.09 ± 2.43 (Day 16)	0.57 ± 0.03	0.41 ± 0.00
4	4	0	30.55 ± 2.54 (Day 19)	82.81 ± 2.65 (Day 21)	0.50 ± 0.06	0.41 ± 0.01
Mineral medium						
5	7	0	4.92 ± 0.77 (Day 0)	452.88 ± 46.85 (Day 7)	0.64 ± 0.08	0.80 ± 0.03

now that the EU has allowed CO_2 for the organic cultivation of microalgae [65], it will be more feasible to cultivate organic microalgae. Secondly, using (a single) plant juice removes the problem with the presence of animal by-products in many waste streams. Many waste streams contain elements, such as animal by products, that prohibit the use of such a stream for the cultivation of algae when feed (or food) applications are desired for the algal biomass [64,66,67]. Thirdly, if the *Chrysanthemum* juice maintains a stable $NH4^+$-N content after and/or during long term storage, one does not always need to recalculate the volume needed to add to the cultivation to obtain the right concentration of nitrogen. Using (mixed) waste streams often comes with the downside of changing nutrient compositions [35,36], an obstacle that could potentially thus be avoided using this *Chrysanthemum* juice. However, further tests with more batches and longer-term storage tests of the *Chrysanthemum* juice are needed to confirm our results.

3.4. Quality and safety of the algal biomass cultivated at pilot-scale

To assess the nutritional quality of the algal biomass cultivation using *Chrysanthemum* juice a Weende analysis was performed (Table 6).

The composition of the obtained algal biomass cultivated in the *Chrysanthemum* juice showed a lower protein content (46–58%) compared to the algal biomass from the positive control test (67%). However, the protein content from the algae cultivated using the *Chrysanthemum* juice was still comparable to previously reported studies on *Tetradesmus* sp. (20–64%) [3,25,68]. The lipid content was similar or slightly higher in the *Chrysanthemum* juice condition (16.57–18.21%) than the positive control (16.34%). This is also comparable to what is reported in the literature for *Tetradesmus* sp. (8–14%) [3,69–72], although higher percentages up to 42–53% have also been described [71,73]. The obtained starch content (0.84–1.79%) in the *Chrysanthemum* juice condition was similar to Cabrita et al. (2022) (1%) [74] or a lot lower compared to the 12.25% obtained by Neha et al. (2025) [72].

Table 6
Composition of the algal biomass after cultivation in the Chrysanthemum juice and mineral medium at pilot-scale. Data are based on the dry weight, except for the moisture content which is based on the fresh algal biomass. P stands for the period.

Weende analysis (g/100 g)	*Chrysanthemum* juice[a]			Mineral medium
	P1	P3	P4	P5
Moisture content	76.1	72.0	75.0	79.8
Ash content	3.56	2.36	2.68	4.01
Total protein (6.25[b])	57.74	46.07	51.20	66.83
Total protein (4.78[b])	44.16	35.24	39.16	51.09
Starch	0.84	1.79	1.60	2.48
Crude fiber	8.79	8.57	8.80	11.88
Total lipids	16.57	18.21	16.80	16.34

[a] For period 2 there was no analysis performed on the biomass.
[b] Both the conversion factors 6.25 and 4.78 were used to provide an upper and lower limit for the total protein content.

Both also studied *T. obliquus*. The obtained crude fiber was lower in the *Chrysanthemum* juice condition (8.57–8.80%) compared to the mineral medium (11.88%) but still alike the reported crude fiber content in the literature (3–10%) [75]. In general, the results obtained by cultivating *T. obliquus* in *Chrysanthemum* juice are thus in line with what is reported in the literature. However, due to large variations it is difficult to compare our results especially given that the composition depends on the medium used, specific algal strain and how the algae were cultivated [76].

To assess the safety of the algal biomass the microbial safety and presence of heavy metals was analyzed (Table S1). Pb and Cd were not detected in any of the tested samples. Cr was detected in all samples cultivated; however, the results show that lower concentrations were observed in the algal biomass cultivated in the *Chrysanthemum* juice medium (between 1.80 and 2.57 μg/mg) compared to the biomass grown in the mineral medium (4.03 ± 0.49 μg/mg). Currently there is no legal threshold for Cr in feed in the EU.

Next to the heavy metals, the microbial quality was also tested. Table 7 shows the evolution of the micro-organisms tested during the cultivation in the bioreactor.

While the Enterobacteriaceae were already present from the start in the photobioreactor, ranging from 100 to 7100 CFU/mL in the liquid culture, which is above the Feed Chain Alliance (FCA) standard [77], *E. coli* was however not detected (noted as <1). The total aerobic cell count was relatively high, however only limited presence of coagulase-positive staphylococci, yeast and fungal cells was observed (respectively, <10 CFU/mL; max 650 CFU/mL and max 560 CFU/mL), below the thresholds of the FCA standards [77]. The increased yeast and fungi during the cultivation could be a result of adding new *Chrysanthemum* juice to the reactor in combination with a shorter cultivation period for the later periods compared to periods 1 and 2. Important to note is that the water used was not sterile itself and already contained a relative high number of microorganisms e.g., a total aerobic cell count of 2.6E04 CFU/mL and 450 CFU/mL fungi.

For the freshly harvested algal biomass however higher CFU counts, above the allowed FCA standards, were observed for all tested indicated organisms, except for Salmonella and Clostridia (Table 7). The high *E. coli* presence at the end of period 4 could potentially be due to a contamination during the handling of the biomass as no *E. coli* (denoted as <1) was found present in the medium itself at the end of period 4 (Table 6). The higher cell counts for the indicator organisms in the harvested algal biomass are most likely due to their up concentration during the centrifugation of the algae. Furthermore, no further processing of the fresh biomass (e.g., freeze drying or a heat treatment) was performed prior to the microbial analysis. There is no general EU standardized regulation for the microbial load in animal feed from vegetable origin. Yet, for animal feed derived from animal by-products there are limits for Salmonella (absent) and Enterobacteriaceae (300 to 5000

Table 7
Microbial load of the photobioreactor (liquid medium) and the algal paste. P stands for period and in total the cultivation was divided in 4 periods, matching 3 partial harvests of 200 L during cultivation (at days 7, 14 and 19) and 1 total harvest of 340 L at day 23, end of cultivation. For each period the microbial load of the medium for the start and at harvest is provided. Start refers to the reactor after harvesting and diluting the culture with water + new Chrysanthemum juice (12 L) added while end stands for a sample taken right before harvesting. For the paste the microbial load is provided for the harvested biomass in periods 1, 3 and 4 (Chrysanthemum juice condition) and for the mineral (positive control) medium condition (period 5).

Period	Total aerobic count	Enterobacteriaceae	Coagulase-positive staphylococci	Fungi	Yeast	*E. coli*
Liquid samples from photobioreactor itself (CFU/mL)						
Tap water						
/	2.6E04	<1	<100	450	<10	<1
***Chrysanthemum* juice based medium**						
P1 start	9.0E05	2000	<10	<10	<10	<1
End	9.6E05	380	<10	<10	<10	<1
P2 Start	2.1E06	100	<10	<10	190	<1
End	8.0E06	2700	<10	<10	<10	<1
P3 Start	5.2E06	7100	<10	<10	<10	<1
End	2.0E07	1200	<10	450	250	<1
P4 Start	6.4E06	1300	<10	150	650	<1
End	4.3E07	180	<10	560	30	<1
Mineral medium						
P5 start	1.3E05	130	<10	<10	<10	<1
End	1.4E05	1500	<10	70	<10	<1
Fresh algal biomass (CFU/g)[a]						
***Chrysanthemum* juice based medium**						
P1 harvest	3.0E07	<10	<100	29,000	24,000	NT
P3 harvest	8.5E07	26,000	<100	140,000	11,000	NT
P4 harvest	6.2E08	290,000	<100	120,000	17,000	30,000
Mineral medium						
P5 harvest	2.5E08	83,000	<100	2800	7600	NT

[a] The data shown here is based on fresh algal paste harvested by centrifugation. No other treatment (e.g., freeze-drying) was done prior to sampling for microbial growth. *Clostridium perfringens* and Salmonella were also tested at 2 time points for the *Chrysanthemum* juice medium test but were never detected. Similarly, for the mineral medium both were never detected. NT: not tested, *E. coli* was not tested for the P1 and P2 harvested biomass. A < 1 result means no cells grew on the petri plates.

CFU/mL depending on the application) (Regulation EC 142/2011) [78]. However, the FCA standards do provide limits for fungi (10,000 to 100,000 CFU/g depending on the application); Enterobacteriaceae (1000 CFU/g), Clostridia (1000 CFU/g) and Salmonella (absent). Most of these limits however apply to thermically treated products [77,79] while we measured the microbial load for fresh algal biomass. To lower the microbial load using *Chrysanthemum* juice for potential commercial applications, a filtration step prior to using the *Chrysanthemum* juice can be implemented, but potentially beneficial effects of bacteria-algae interactions might be removed. Furthermore, a thermal treatment of the harvested algal biomass could also lower the microbial load. Generally, postharvest treatments of fresh products will lower the microbial load [80]. Another important remark is that the Enterobacteriaceae and total aerobic count in the *Chrysanthemum* juice test experiment were potentially higher due to the sampling of the *Chrysanthemum* juice during storage. For sampling, the storage container had to be opened to manually take samples from the top of the container. This could have had an effect on the microbial load in the *Chrysanthemum* juice as opening the container introduced oxygen and microorganisms into the container, distorting the microbiome already present in the juice. This was visible by a microbial biofilm that formed on top of the juice after sampling started. This biofilm layer was originally absent after initially receiving the container with the *Chrysanthemum* juice. However, while the algae cultivation in the mineral medium contained less yeast and fungi, it also contained in general high amounts of indicator organisms, especially for the total aerobic cell count and the Enterobacteriaceae (Table 7). This might indicate that the high microbial load for the algal biomass cultivated in the *Chrysanthemum* juice based medium was not solely due to the use of the *Chrysanthemum* juice. This points towards the need to introduce more stringent measurements to lower the microbial load and to perform post-treatments of the harvested biomass.

In future experiments, one could use a system in which one does not have to open the container to take a sample and as such avoid contact with the *Chrysanthemum* juice while keeping the *Chrysanthemum* juice under anaerobic conditions inside the container. However, given the high microbial presence in the positive control, it also points towards a general presence of micro-organisms in the tap water used.

4. Conclusion

An algal biomass composition comparable to previously reported data was achieved, demonstrating the potential of *Chrysanthemum* juice as a nutrient source for the cultivation of microalgae as a feedstock. However further tests are required to assess the safety and quality of the algal biomass. Particularly, depending on the specific application a more in-depth analysis of the algal biomass composition is required. To increase the growth and final dry weight yields further cultivation optimization steps are required. Also, extra postharvest treatments of the algal biomass and potentially filtration of the *Chrysanthemum* juice (and water) prior to use should be investigated in order to reduce the microbial load of the biomass to meet the criteria for animal feed. From the tested heavy metals, Pb, Cd and Cr, only the latter was detected in the algal biomass, however, currently the EU has not yet set a limit for the presence of Cr in feed. Future studies should investigate more heavy metals for their potential presence in the algal biomass to have a more in-depth idea on their presence in the algal biomass. Furthermore, future studies should also check for the presence of specific pesticides in the algal biomass itself as we only checked for their presence in the fermented *Chrysanthemum* juice and found three of them, two fungicides mepanipyrim and flonicamid and the insecticide sulfoxaflor, to be above the legal threshold in the EU. Lastly, a more in-depth study of the evolution of the *Chrysanthemum* juice during storage and the use of a closed system with a drain system to prevent external influences when sampling juice. Nonetheless, this proof-of-concept pilot-scale study shows the feasibility of using *Chrysanthemum* juice for the cultivation of microalgae.

CRediT authorship contribution statement

Floris Schoeters: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Audrey De Cuyper:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tom Bernaerts:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Laura de Kluijver:** Writing – review & editing, Investigation. **Sarah Goossens:** Writing – review & editing, Investigation. **Charl Goossens:** Writing – review & editing, Investigation. **Sabine Van Miert:** Writing – review & editing, Conceptualization.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

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Declaration of competing interest

VARTA is a commercial entity that works on the commercialization of products derived from agricultural waste.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2026.104575.

Data availability

Data will be made available on request.

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