Towards sustainable water management for Galdieria sulphuraria cultivation

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Towards sustainable water management for *Galdieria sulphuraria* cultivation

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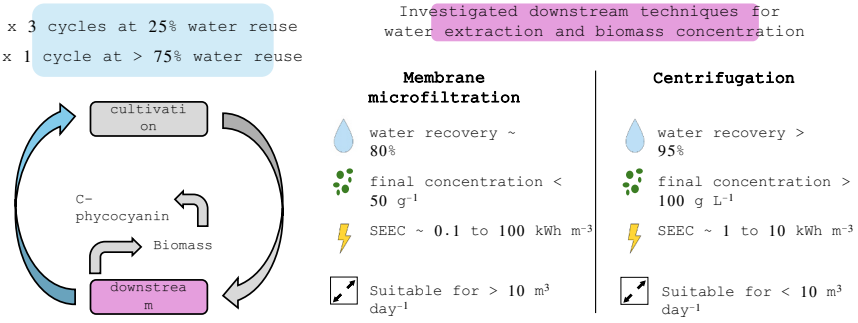
HIGHLIGHTS

- *Galdieria sulphuraria* cultivation necessitates management of acidic wastewater.
- Water and nutrient reuse enhance the sustainability of the cultivation process.
- 25% water reuse maintains biomass productivity (~ 0.21 g L^{-1} d^{-1}) and phycocyanin accumulation (~10.8% *w*/w).
- High water reuse (71%, 95%) does not affect productivity, equal to 0.24 g L^{-1} d^{-1} in a single reuse cycle.
- Energy needs of centrifugation and membrane filtration depend on system size and biomass concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

The red microalga *Galdieria sulphuraria* has emerged as a promising biotechnological platform for large-scale cultivation and production of high-value compounds, such as the blue pigment phycocyanin. However, a large amount of freshwater and a substantial supply of nutrients challenge both the environmental and the economic sustainability of algal cultivation. Additionally, the extremophilic nature of *Galdieria sulphuraria* requires cultivation in an acidic culture medium that directly leads to strongly acidic wastewater, which in turn generally exceeds legal limits for industrial wastewater discharge. This research aims to address these challenges, by investigating cultivation water reuse as a strategy to reduce the impacts of *Galdieria sulphuraria* management. The results indicated that a 25 % water reuse may be easily implemented and showed to be effective at the pilot scale, providing no significant changes in microalgae growth (biomass productivity ~0.21 g L^{-1} d^{-1}) or in phycocyanin accumulation (~ 10.8 % *w*/w) after three consecutive cultivation cycles in reused water. Moreover, a single cultivation cycle with water reuse percentages of 71 and 98 %, achieved with membrane filtration and with centrifugation, respectively, was also successful (biomass productivity ~0.24 g L^{-1} d^{-1}). These findings encourage freshwater reuse implementations in the microalgae sector and support further investigations focusing on coupling cultivation and harvesting in continuous, real-scale configurations. Centrifugation and membrane filtration required substantially different specific electrical energy consumption for water reuse and biomass concentration: in real applications, the former technique would roughly span from 1 to 10 kWh m^{-3} while the latter is expected to fall within the ample range 0.1–100 kWh m^{-3}, strongly dependent on system size. For this

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reason, the most suitable separation train should be chosen on a case-by-case basis, considering the prevailing flow rate and the target biomass concentration factor targeted by the separation process.

1. Introduction

Microalgae cultivation has become increasingly popular due to its potential across various industries (Spolaore et al., 2006). As a result of CO_2 fixation, microalgae are capable of accumulating significant amounts of carbohydrates, proteins, lipids, and other valuable compounds, such as pigments and vitamins, making them a promising energy feedstock with versatile applications in the production of dietary supplements, cosmetics, food, animal feed, and biofuels (De Luca et al., 2021; Gimpel et al., 2015; Nędzarek and Mitkowski, 2022; Xu et al., 2023). However, an important challenge associated with microalgae cultivation is, among others, the large amount of water required for biomass production, as well as the continuous replacement of freshwater in photobioreactors to support the biological functions and growth of algae. The high cost of freshwater, in terms of energy and environment impact, and its limited availability, especially in water-stressed regions, have contributed to impairing the growth of the microalgae industry (Suparmaniam et al., 2019). Optimizing the harvesting process and investigating the feasibility of reusing water would lead to the reduction of environmental and management costs associated with microalgae cultivation (Wu et al., 2021), moving towards a circular economy approach.

The polyextremophile red microalga *Galdieria sulphuraria* has gained extensive attention for its ability to survive in harsh conditions, such as low pH (as low as 0.2 for some strains) (Abiusi et al., 2021), elevated temperatures (up to 57 °C), and high osmotic pressure (Pinto et al., 2007). *G. sulphuraria* has been found to be a rich source of proteins, insoluble dietary fibers, and antioxidants (Carfagna et al., 2016; Graziani et al., 2013). It also contains a high proportion of essential sulphur amino acids compared to other sources, *e.g.*, *Chlorella*, *Spirulina*, and soybean protein (Abiusi et al., 2022). Its blue-green color is attributed to the presence of blue phycobiliproteins C-phycocyanin (C-PC) and allophycocyanin, as well as chlorophyll *a*. Furthermore, the C-phycocyanin extracted from *G. sulphuraria* is more stable at low pH and high temperatures than that extracted from *Arthrospira platensis*, the latter representing the nearly exclusive *C-PC* production platform today. These characteristics position *G. sulphuraria* as a promising candidate for large-scale production as a food and feed source.

Since *G. sulphuraria* cultivation medium requires low pH conditions, sulphuric acid is commonly added to the cultivation medium, leading to highly acidic wastewaters, which exceed typical wastewater discharge standards, including Italian limits for industrial wastewater discharge after biomass harvesting (Annex 5, Third Section, Legislative Decree n. 152/2006 (Italian Legislative Decree No. 152 approving the Code on the Environment, n.d.)). Therefore, the aim of this work is to assess two separation techniques, namely, centrifugation and membrane microfiltration, in their ability to extract water to be reused as new cultivation medium. In particular, different fractions of reused water are investigated, as well as their effects for one or multiple cycles of *G. sulphuraria* cultivation at the pilot scale. Additionally, the potential *C-PC* content achievable within the employed photobioreactor (PBR), under control conditions (distilled water plus salts) as well as when algae are grown in reused water, is discussed. Finally, a comparison between centrifugation and membrane filtration is provided in terms of achievable extraction and energy input at different scales, with the goal to provide a preliminary guide towards the choice of the most suitable water reuse technique.

2. Materials and methods

2.1. The planar photobioreactor

The flat-panel PBR used in this work has been described in the literature (Carone et al., 2022), and was recently upgraded with a LEDs artificial light source in place of fluorescent tubes, allowing for less energy consumption and better regulation of specific wavelength requirements for the selected microalgae species (Carone et al., 2024). Briefly, the PBR is composed of two interconnected units: a photo-stage loop and a mixing tank. The photo-stage loop consists of two parallel alveolar flat panels illuminated by an interposed optical guide which redirects the light coming from two LEDs rods arranged at the top and at the bottom of the guide, perpendicularly to the panels. The alveolar flat panels are made of transparent polycarbonate with an exposed surface area to light of 1.5 m^2 each and an internal path of 13 mm, for a total volume of 17 L. The mixing tank is made of a darkened HDPE (high-density polyethylene) material with a total useful volume of 50 L. A hydraulic circulator is connected at the bottom of the mixing tank and upstream of the photo-stage loop, driving the liquid flow into both flat panels, from the bottom to the top. Temperature, pH, dissolved oxygen, and carbon dioxide are constantly monitored by specific sensors (Mettler-Toledo, USA) located at the output of the flat panels. The signals from the sensors are transmitted, through a multi-parameter transductor, to a programmable logical controller (PLC, Unitronics, Israel) for data storage, online monitoring, and control.

2.2. Microalgae growth and cultivation conditions

Galdieria sulphuraria strain 074 W was kindly donated by Prof. Antonino Pollio (University of Naples, Italy). All the experiments were conducted in batch mode and axenic conditions under constant artificial illumination with specific light spectra according to previously published results (Carone et al., 2024). The PBR was inoculated with: (i) Allen medium (control conditions) acidified at pH 2 with sulphuric acid (Allen, 1959), or with (ii) recovered water added with distilled water in a ratio 1:3 (with nutrients reintegration), or with (iii) reused water only (with nitrogen and phosphorous reintegration), as well as with microalgae cells, reaching a total volume of 45 L. The initial biomass dry weight was about 0.25 g L^{-1} for all the tests. The injection of CO_2 (food grade 99.9 %) was carried out with a flow rate of 0.06 NL min^{-1}, keeping constant the CO_2 concentration threshold in the PBR at 15 mg L^{-1} using a combination of solenoid valve and mass flow meter. The cultures were maintained at a constant temperature of 37 °C ± 2, and under constant artificial illumination (averaged 120 μmol m^{-2} s^{-1}). Microalgae growth was gravimetrically quantified as dry biomass concentration as previously reported (Carone et al., 2022). The averaged biomass productivity (P_x, g $L^{-1}d^{-1}$) was calculated as:

$$P_x = \frac{X_t - X_0}{t - t_0} \quad (1)$$

where X and X_0 are the final and initial concentrations (g L^{-1}), respectively, and $t - t_0$ is the time passed between the two measurements.

2.3. Microalgae harvesting methods

Cells reaching the stationary phase were collected and centrifuged using a CLARA-20 centrifuge (Alfa Laval, Sweden) model operated with a starting flow of 100 L h^{-1} up to150 L h^{-1}, and a counter pressure of about 1.8–1.9 bar. On the other hand, for what concern the harvesting through the microfiltration process, a standard system configuration

was employed. A TiO_2 ceramic membrane (TAMI industries, France) with 0.14 μm pore-size was selected as it proved its effectiveness among other microfiltration and ultrafiltration membranes when concentrating algal biomass in the same concentration range (Malaguti et al., 2023; Ricceri et al., 2022). The tubular membrane length was 1170 mm in length and its active filtration area was 0.21 m^2. Two different filtration protocols were performed: the first one consisted in semi-batch operations whereby, at fixed recovery rate values, the permeated water was recirculated into the feed tank until steady-state conditions were reached and then the loop was opened to separately collect the permeate water. This protocol was repeated at the following five recovery values: 0, 25 %, 50 %, 75 %, and 90 %. In the second protocol, the permeated water was collected separately and continuously, thus operating in an open loop configuration: in this case, the feed solution was continuously concentrated until the highest possible recovery rate was reached. For both testing protocols, the same operating conditions were used: the cross-flow velocity was 2.5 m s^{-1}, corresponding to a feed flow rate of 1.9 m^3 h^{-1}, while the average trans-membrane pressure was 1.6 bar.

The concentrated biomass (approximatively 2 L), either from centrifugation or microfiltration, was frozen at −85 °C and subsequently lyophilized (ScanVac CoolSafe Touch 55–4 Freeze Dryer, LaboGene, Denmark) to facilitate further extractions. As mentioned above, the reused water was either mixed with distilled water (with nutrient reintegration) in a 1:3 ratio (25 % reused water and 75 % distilled water), or used as is (~98 %, with nutrients reintegration) to prepare the cultivation medium for subsequent algae growth.

2.4. C-PC extraction and quantification

The *C-PC* from *G. sulphuraria* was quantitatively extracted by bead beating (Mixer Mill MM 400, Retsch, Germany) approximatively 1 g of lyophilized biomass. Lyophilized cells were resuspended in 100 mM Na-phosphate buffer at pH 7 and exposed to 3 × 5 min beating cycles at a frequency of 30 Hz with 5 min breaks on ice between each cycle. Cell debris was removed through centrifugation at 16,000 rpm for 10 min and the supernatant was collected in fresh tubes. This extract is called crude extract. The *C-PC* contents were calculated measuring the absorbance at 620 and 652 nm and converting the measured absorbance to concentration using the Kursar and Alberte equation (Kursar and Alberte, 1983).

2.5. Macro- and micro-nutrients monitoring

Macronutrients and micronutrients were quantified after water extraction and, if needed, re-integrated in the solution to achieve the same concentrations of the ideal Allen medium. Nitrogen (N) and phosphorous (P) were quantified spectrophotometrically (Onda UV-31 Scan spectrophotometer, China) using standard reagent kits for sensitive photometric measurements (NANOCOLOR test kit, Macherey-Nagel, Germany). All the other metals, namely, magnesium (Mg), potassium (K), manganese (Mn), sodium (Na), iron (Fe), cobalt (Co), and molybdenum (Mo), were quantified with inductively coupled plasma metal analysis (OPTIMA 2000 ICP optical emission spectrometer, PerkinElmer, U.S.A.). Water samples were filtered using 0.45 μm filters prior to analysis with ICP. Three calibration curves, each containing different mixtures of metals, were prepared as follows: Mg, Na, and K at concentrations of 12.5, 25, 50, and 100 mg L^{-1}; Fe and Mn at concentrations of 1, 2, 4, and 8 mg L^{-1}; Mo and Co at concentrations of 0.08, 0.16, 0.32, and 0.64 mg L^{-1}. The alkali metals (Mg, Na, K) were measured with the torch oriented radially, while the other metals (Fe, Mn, Mo, Co) were measured with the torch oriented axially.

3. Results and discussion

3.1. Microalgae cultivation in partially reused water (25 %)

In recent years, *Galdieria sulphuraria* has emerged as a promising biotechnological platform for large-scale cultivation and production of a high-nutritional value biomass, for nutraceutical purposes, as well as to produce high-value molecules, such as the blue pigment phycocyanin. However, being an extremophilic species, it requires cultivation at high temperatures, and most importantly, in an appropriately acidified culture medium (Cheng et al., 2019). The acidification of the medium is commonly achieved using sulphuric acid, which results at the end of the process and after biomass harvesting in a strongly acidic wastewater outside the legal limits for industrial wastewater release (Thielemann et al., 2021). Moreover, since the use of a large amount of freshwater is among the main costs associated with large-scale algae cultivation, the possibility of reusing water and thus recycling metals for multiple cultivation cycles would significantly reduce costs. In this study, a 25 % water reuse factor was first targeted to assess the preliminary feasibility of the process. In fact, the reused water was always characterized by a yellowish color due to the likely presence of algae organic matter (*AOM*), which, without appropriate dilution, might lead to a strong attenuation of light, interfere with algae growth, and/or contribute to biofouling within the PBR (Sha et al., 2019). After an initial batch cultivation with standard (control) medium, 3 consecutive cycles of harvesting and re-inoculation using the reused water mixed with distilled water in a ratio of 1:3 were carried out. This experiment was conducted identically with the reused water obtained from centrifugation and from membrane filtration. Note that the harvesting processes were conducted starting from a biomass concentration of 1 g L^{-1}, achieved after algae growth in each of the cycles. For these tests, only N and P concentrations were quantified in the reused water and re-integrated in each of the cycles to achieve the starting, ideal concentrations, equal to those of the standard solution. All the other salts were added to the final working volume without prior measurement, according to the medium recipe. This protocol was adopted because dilution would have reduced the concentrations of most micronutrients (Mo, Co, Mn, Fe) likely below the detection limit, and also to prevent possible limiting conditions on the microalgal metabolism. Despite the dilution, given the high content of protons due to the first acidification with H_2SO_4 and the lack of a buffer system, the pH remained between 3 and 3.5 for all the cultivation cycles, therefore no pH adjustment was performed.

Fig. 1 reports the *G. sulphuraria* growth data in control conditions and in each consecutive cycle of growth in partially reused water derived from the centrifugation (Fig. 1A) and membrane microfiltration (Fig. 1B) processes. After 16 days of cultivation, the biomass concentration reached 3.26 g L^{-1} ± 0.15 in control conditions, with an average biomass productivity (P_x) during the exponential phase of 0.21 g L^{-1} d^{-1} ± 0.06. Growth in partially reused water showed negligible differences with respect to the control condition for both the downstream processes employed, reaching the same final concentrations at the end of the cultivation period. Indeed, the average P_x during the exponential phase was 0.22 ± 0.10, 0.20 ± 0.07, and 0.20 ± 0.05 g L^{-1} d^{-1}, for the three cycles with centrifugation, respectively, and 0.22 ± 0.02, 0.24 ± 0.04, and 0.21 ± 0.03 g L^{-1} d^{-1}, respectively, for the membrane microfiltration process. For all the batch cultivations, the dissolved O_2 reached a steady state concentration of 23–24 mg L^{-1} after 6–7 days of cultivation, indicating that *G. sulphuraria*'s photosynthesis was not impacted by the reused water in any of the three cycles.

The results reported in Fig. 2 delve into the performance of the microfiltration process. The water flux across the membrane decreased from the first to the third reuse cycle, with a trend suggesting a diminishing rate of reduction. As expected, increasing the water recovery, in turn leading to increased biomass concentration in the feed solution, translated into a decrease of water flux and increase in fouling. The release of AOM consequent to algal cell break and the possible

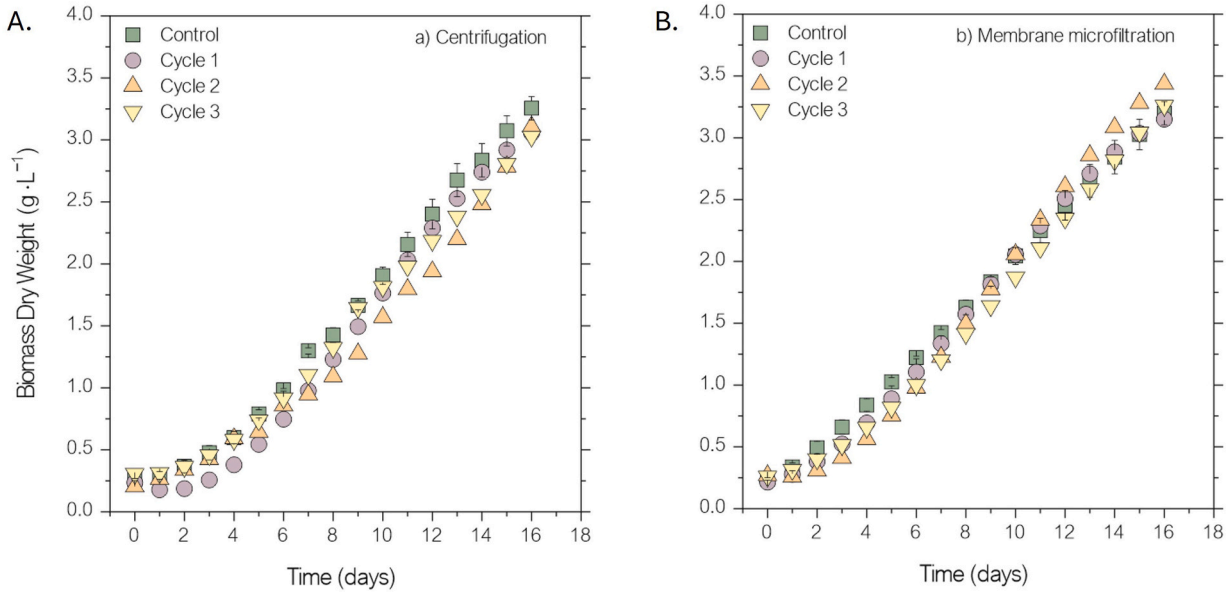

Fig. 1. Biomass concentration measured over time during cultivation. A. Cycles of partially reused water from centrifugation. B. Cycles of partially reused water from membrane microfiltration. Green squares: control ($n = 3$). Purple circles: 1st cycle at 25 % water reuse. Orange up-pointing triangles: 2nd cycle at 25 % water reuse. Yellow down-pointing triangles: 3rd cycle at 25 % water reuse.

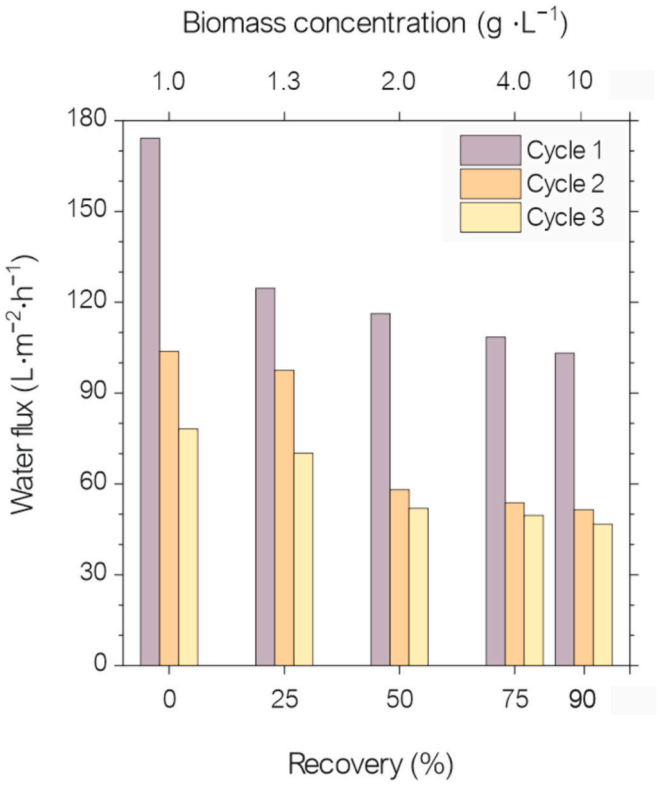

Fig. 2. Water flux across the microfiltration membrane as a function of water recovery values. The reported water flux values were measured upon reaching flux stabilization for each recovery value. These results refer to a starting biomass concentration of 1 g L^{-1} and an applied pressure of 1.6 bar.

accumulation of AOM from one cycle to the next translated into more important clogging of the membrane pores, likely due to low MW compounds (Mkpuma et al., 2022). These results suggest that multiple water reuse cycles can be potentially achieved by membrane filtration, but with a reduction of membrane productivity, hence harvesting efficiency. That being said, the observed flux was always above 40 L m^{-2} h^{-1}, even in the third reuse cycle and at 90 % recovery rate (10× algae concentration factor in the feed suspension, equivalent to a cell concentration of 10 g L^{-1}). The flux results suggest that it should be possible to maintain a minimum flux larger than ~30–40 L m^{-2} h^{-1} and an average flux larger than 40–50 L m^{-2} h^{-1} for several reuse cycles and working at algae cell concentrations between roughly 1 and 10 g L^{-1}. In the last filtration cycle shown in Fig. 2, the dissolved organic carbon (DOC) concentration in the permeate water was measured at each recovery step: 0, 25 %, 50 %, 75 %, and 90 % recovery values corresponded to concentration of DOC of respectively 52.6 ppm, 54.7 ppm, 55.3 ppm, 58.8 ppm, and 60.3 ppm. These results highlighted a trend: DOC concentration increased in the permeate as feed concentration increased, consistent with theoretical expectations assuming a constant membrane rejection, which results in increased permeation with increased concentration in the feed suspension.

3.2. Microalgae cultivation with highest recovered water volume

Appraised the potential of partial water reuse, both in terms of algae cultivation in reused media and harvesting process, full water reuse was assessed while simultaneously stressing the harvesting system by using suspensions with initial biomass concentration equal to 4 g L^{-1}. In particular, the centrifugation allowed retrieving 99 % of the total water volume (44.55 L out of 45 L of water were recovered), of which 44 L were used as new culture medium (~98 %) upon mixing with 1 L (~2 %) of fresh microalgae inoculum. Therefore, a concentration factor of ~100 was achieved by centrifugation, reaching algae concentrations of roughly 400 g L^{-1} in the final concentrated slurry. Whereas, by concentrating the microalgae substrate with membranes, the achieved water recovery was approximately 80.5 % (32.2 L out of 40 L of water were recovered), corresponding to a 5.1 concentration factor and a final algae concentration of approximately 20.5 g L^{-1} in the concentrate stream. Fresh microalgae inoculum and additional distilled water were then added in order to reach the final working volume of 45 L, corresponding thus to an overall 71.5 % of reused water. For these tests, only one cycle of water reuse was assessed on independent trials for the two downstream processes. All the nutrients were quantified in the reused water; see Table 1. Since the concentration of all the monitored metals

Table 1
Concentration of metals, ions, and pH value in the culture medium in different solutions, before and after cultivation, before and after water recovery with centrifugation or microfiltration.

Metal/ion	Concentration in the Allen medium (control) [mg L^{-1}]	Concentration after biomass cultivation pre – centrifugation [mg L^{-1}]	Concentration in the recovered water from centrifugation [mg L^{-1}]	Concentration of the feed solution prior to membrane microfiltration [mg L^{-1}]	Concentration in the permeated water from membrane microfiltration [mg L^{-1}]
Mo	2.38 ± 0.02	1.13 ± 0.03	1.126 ± 0.026	2.20 ± 0.07	1.60 ± 0.05
Co	0.028 ± 0.00	0.03 ± 0.00	0.029 ± 0.00	0.06 ± 0.02	0.05 ± 0.03
Fe	2.223 ± 0.021	1.90 ± 0.03	1.904 ± 0.027	1.46 ± 0.04	1.36 ± 0.05
Mn	2.285 ± 0.011	2.02 ± 0.05	2.019 ± 0.047	2.11 ± 0.03	2.01 ± 0.02
Mg	39.35 ± 0.042	33.02 ± 0.69	33.02 ± 0.684	26.96 ± 0.74	12.91 ± 0.66
Na	10.73 ± 0.24	11.09 ± 0.28	11.088 ± 0.282	11.30 ± 0.34	11.44 ± 0.22
K	101.2 ± 0.707	67.79 ± 1.39	67.793 ± 1.392	58.87 ± 2.11	56.15 ± 2.98
NH_4^+	470.7 ± 2.22	47.55 ± 0.84	47.55 ± 0.84	n.d.	n.d.
PO_4^{3-}	250.10 ± 1.46	147.18 ± 4.25	147.18 ± 4.25	143.55 ± 5.41	142.88 ± 4.44
pH	2.00	1.95	1.94	1.93	1.93

did not decrease significantly, indicating an excess of nutrients in the ideal medium, only N and P were re-integrated into the reused water. As expected, the pH values of reused water in the final working volume were found to be 2 and 2.6, for the centrifugation and membrane microfiltration experiments, respectively, and therefore no pH adjustment was required.

The results displayed in Fig. 3 indicate that *G. sulphuraria* growth was not affected by the use of the maximum recoverable water from both the downstream processes. The averaged P_x during exponential growth was 0.25 ± 0.08, 0.24 ± 0.06, and 0.24 ± 0.10 g L^{-1} d^{-1} for the control batches and the growth on reused water from the centrifugation and membrane microfiltration processes, respectively. Despite the strong yellowish color of the reused water, indicating the likely presence of a substantial amount of *AOM*, no differences in growth rate or in terms of biofouling were observed in one cycle of water reuse. Also in these experiments, the dissolved O_2 reached a steady state concentration of 22 mg L^{-1} after 7 days of cultivation. Further experiments are necessary to address the feasibility of reusing water for more consecutive cycles and a proper integration of nutrients according to the microalgae needs and according to economic criteria (Lu et al., 2020). Note that no substantial loss of micronutrients was observed upon centrifugation or microfiltration, with measured metal and ion concentrations close to the values determined in the cultivation suspension upon biomass growth in the ideal Allen medium (Table 1). Only a certain loss of magnesium was observed in the microfiltration test, which may be simply related to experimental error and would require further investigation.

Results in Fig. 4 delve into the microfiltration behavior when separating water from a feed stream containing an initial biomass concentration of 4 g L^{-1} in open-loop configuration, *i.e.*, permeate stream continuously recovered externally to the feed loop until the maximum recovery achievable in the employed system was reached (80.5 %). The water fluxes were consistent with those reported in Fig. 2. In particular, the system started with a flux roughly equal to 72 L m^{-2} h^{-1}, which is within the range of flux values observed at the recovery rate of 75 % in the experiments starting with a biomass concentration equal to 1 g L^{-1}. The flux decreased to a value of 55 L m^{-2} h^{-1} at the end of the filtration, once again suggesting the feasibility of the microfiltration system to concentrate biomass and extract freshwater, at least in terms of system productivity. DOC and TN were measured in the initial feed stream, in the concentrate stream, in a permeating water sample collected in the beginning of the experiment, and in the total permeated volume. DOC results were 216 ppm, 411 ppm, 140 ppm, and 169 ppm respectively,

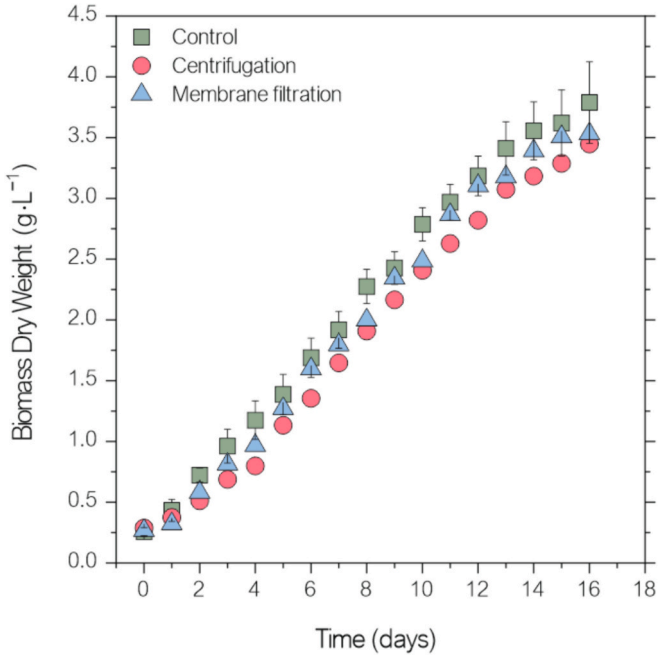

Fig. 3. Biomass concentration over time during cultivation in reused water. Green squares: control ($n = 3$). Red circles: growth in 98 % reused water from centrifugation ($n = 2$). Blue triangles: growth in 71.5 % reused water from microfiltration.

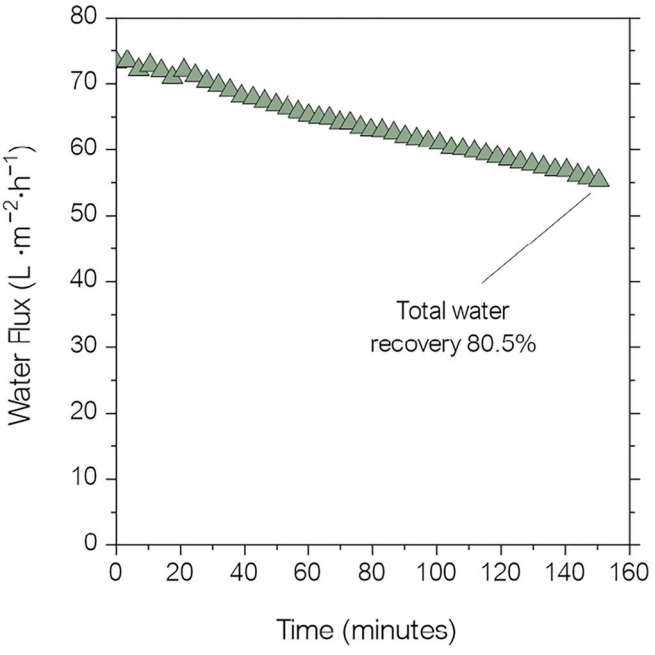

Fig. 4. Measured water flux (y-axis) during microfiltration reported against time (x-axis). These results refer to a starting biomass concentration of 4 g L^{-1} and an applied pressure of 1.6 bar.

while TN concentrations were respectively equal to 24.4 ppm, 44.0 ppm, 18.0 ppm, and 22.6 ppm. The DOC and TN rejections provided by the membrane were thus roughly 35 % and 20 %, respectively. However, the system rejections, calculated from the concentrations in the total permeated volume with respect to the initial feed, were approximately 20 % and 5 %, respectively. Note that the membrane pores are much larger than any dissolved substance and that filtration operated by the algae cake is likely responsible for the majority of the observed rejection of DOC and TN. Differences in the absolute DOC concentration values between the partial and full water reuse scenarios were due to the different initial algal concentration (1 g L^{-1} and 4 g L^{-1}, respectively).

3.3. Analysis of energy consumption for water reuse

In this study, two different downstream processes for biomass separation were employed, namely, microfiltration and centrifugation. Among the wide spectrum of possible concentrating techniques, these processes represent the most adopted solutions for biomass separation from the liquid phase as of today (Zhao et al., 2020). A direct energy comparison between the two techniques is far from straightforward, due to the fundamental difference in their separation mechanism, the variety of conditions, and the multiple parameters affecting energy expenses. However, two variables that are hypothesized to influence the energy performance of concentration systems and, therefore, the choice of the most suitable harvesting option, are the following. (i) Cultivation volumes or flow rates: energy expenses are related to the size of the system, often in a non-linear and complex way. (ii) Starting and final biomass concentrations and the relative concentration factor. This discussion aims at reviewing energy expense figures related to the two separation techniques in the light of the two variables just highlighted, placing energy figures into context, and drawing conclusions that may guide a rational choice of the most suitable process aimed at biomass harvesting and freshwater extraction.

(i) To understand the effect of system size, the discussion starts with reviewing the energy expenses measured with the lab-scale units utilized in this work, then those that are estimated for efficient large-scale systems, and will finally provide ranges associated pilot-scale units and reported in the literature. The membrane separation system and the centrifuge deployed in this research were laboratory-scale units, far from being optimized in terms of energy consumption. The measured, specific electrical energy consumption (SEEC) of the membrane separation was approximately 25 kWh m^{-3} (energy needed for each m^3 of extracted freshwater), calculated by simply considering that the power absorbed by the pump was ~0.3 kW and that the system recovered 32.2 L of water out of 40 L of diluted biomass in 150 min of operation. On the other hand, the separation operated with the centrifuge was associated with a SEEC of approximately 14 kWh m^{-3}, calculated considering that the power absorption of the system was ~2.2 kW and that it separated 39.2 L of water out of 40 L of diluted biomass in 15 min. It is worth highlighting again that these figures do not represent those that would be necessary in a real scale plant, but they serve the goal of highlighting the importance of system scale and modularity features.

On the other end of the spectrum compared to lab-scale systems, large-scale plants aim at reducing irreversibility issues, thus using energy in an efficient way and approximating as much as possible the energy of separation that can be estimated from first principles. For example, the SEEC expected for an efficient membrane-based separation driven by applied pressure can be calculated directly starting from the Bernoulli's principle as follows:

$$SEEC_m = \frac{Q_{feed} \bullet (\Delta P + \Delta P_{loss})}{Q_{perm} \bullet \eta \bullet 36} \quad (2)$$

where Q_f is the feed flow rate (L h^{-1}), Q_{perm} is the permeate flow rate (L h^{-1}), η is the efficiency of the pump (−), assumed equal to 0.5 to give a conservative estimation, ΔP is the applied pressure (bar), and ΔP_{loss} represents the pressure losses (bar) that can be conservatively assumed equal to 0.1 bar for each meter of membrane module. The ratio between Q_{perm} and Q_f represents the single pass recovery rate of the system that proved to be easily above 80 % for the application of this study. The resulting theoretical SEEC value for the extraction of freshwater, assuming the same conditions of the laboratory unit utilized in this study (ΔP = 1.6 bar, ΔP_{loss} = 0.12 bar relative to a 1178 mm long module), is 0.12 kWh m^{-3}. This number is roughly 200 times smaller than the energy value needed with the lab-scale unit utilized in this study and is not far from what would be expected in a large-scale microfiltration system. Indeed, optimized large-scale microfiltration plants for surface water treatment consistently show energy consumptions around 0.3 kWh/m^3 at very high recovery rates (> 95 %).

SEEC values reported in the literature for pilot-scale membrane units operated for biomass harvesting range from 0.97 kWh m^{-3} to 2.5 kWh m^{-3}, one order of magnitude higher than the value estimated from Bernoulli's principle. The former value refers to the optimized conditions of a system managing 2000 L microalgae suspension and a volume concentration factor equal to 200 (Gerardo et al., 2015), while the latter value refers to an experimental measurement of the harvesting of 200 L microalgal suspension reaching a volume concentration factor of 39.2 (Khan et al., 2023). Considering instead available data of commercial centrifuge systems, the Clara 750 and Clara 20 from Alfa Laval may be taken as representative examples. They are associated to SEEC values for centrifugation of 0.9 kWh m^{-3} and 4.4 kWh m^{-3}, respectively. Note that these two centrifuge units represent the limits of systems commercialized by Alfa Laval: Clara 750 operates at a maximum flow rate of 50 m^3 h^{-1}, while Clara 20 at 0.5 m^3 h^{-1}. These SEEC values are in the same order of magnitude as those that can be estimated from equations based on centrifugal forces, applied by some authors to estimate the energy expense of centrifugation starting from first principles (Coons et al., 2014; Najjar and Abu-Shamleh, 2020).

The numbers reported above highlight an important trend. The energy needs of membrane-based microfiltration may span as much as four orders of magnitude (0.1 to 100 kWh m^{-3}) as a function of system size. This ample range is due to the intrinsic modularity of membrane systems: the number of modules in parallel and in series, the overall membrane active area, bypass and recirculating streams, and the possible presence of pressure recovery devices, strongly impact the energy consumption. In this respect, systems with larger membrane active area are characteristically more energetically efficient than smaller ones. Energy values for centrifugation systems fall instead within two orders of magnitude only (1 to 10 kWh m^{-3}), implying that system size plays some role, but substantially less so compared to membrane units. As hypothesized above, the scale of the system is in fact an important guiding principle to select the best downstream harvesting technique.

(ii) When considering the other variable hypothesized above, *i.e.*, achievable biomass concentrations and concentration factor, note that microfiltration has limitations when it comes to the concentration of microalgae that can be attained in the retentate stream, due to issues associated with fouling and/or cake build-up when highly concentrated suspensions are filtered. It is unlikely that a well-operated microfiltration system could reach concentrations substantially larger than ~50 g L^{-1} for the harvested biomass, possibly reaching values in the order of 100 g L^{-1} in the best-case scenario. On the other hand, well-operated centrifugation systems have been shown to achieve considerably larger biomass concentration in the harvested product, at least 150 g L^{-1} (as also observed in this study), but possibly up to an order of magnitude of 1000 g L^{-1}. Biomass contents above 100 g L^{-1} are required by most of the applications seeking to

utilize the harvested biomass for beneficial purposes. Therefore, the majority of biomass harvesting plants would likely require a single or a final concentration step that would guarantee such target biomass concentration values.

Given the assessments discussed above, some rational guiding principles may be formulated. For biomass plants producing $< \sim 10$ m^3 of algae suspension daily, a harvesting system comprising a single centrifugation step would possibly be the best option, since it would allow achieving a target concentration factor while requiring reasonable energy inputs. As a reference scenario for understanding the actual size of real microalgal biomass harvesting systems, the cultivation of Astaxantina is analyzed. Although its cultivation represents a relatively small market, with an average estimated produced volume of 18,500 kg yr^{-1}, five players produce around 72 % of the overall final product. Among these companies, BGG, Algatech, and Algalif currently produce, respectively, ~4000, 2500, and 2500 kg yr^{-1} of final product using PBR cultivation technology, while Cyanotech currently produces ~1500 kg yr^{-1} with open ponds. Assuming typical microalgal concentrations in pond cultivation from 0.5 to 0.8 g L^{-1} and concentrations from PBR in the range 1–2 g L^{-1}, the volumes of diluted microalgal biomass being treated every year range from 1250 to 4000 m^3, corresponding to daily flow rates from 3.4 to 11 m^3. As the biomass cultivation plant increases in size above this range, membrane systems become increasingly competitive in terms of energy consumption, to the point where it would make sense to pre-concentrate the biomass suspension using a membrane-based separation and then achieve the final harvesting target with a centrifugation process. Such a two-step process would combine the intrinsic ability of medium to large-scale membrane systems to effectively extract large volumes of freshwater with relatively low specific energy demands, and the intrinsic ability of centrifuge systems to concentrate the biomass to high values while managing a suspension of smaller volume.

As a disclaimer to the guiding principles just highlighted, the most suitable separation train should be evaluated in each case, also to ensure the continuity between the bioreactor and the harvesting process when the extracted freshwater is to be reused in part or in full. Also, note that the microalgal strain may have an impact on the efficacy of the harvested techniques: different species have shown distinct results when concentrated using the same technique, seen differences in their shape, size, and chemical composition (Ricceri et al., 2022; Suparmaniam et al., 2019).

3.4. Biomass quality and environmental benefits of water reuse

This study indicates the technical feasibility of reusing cultivation water for *Galdieria sulphuraria* growth, potentially yielding significant resource savings and environmental impact reduction. Monitoring water quality throughout the cultivation process, both upstream and downstream, is crucial for effective water reuse and it allows precise interventions, such as nutrient reintegration. In particular, N and P reintegration may be optimized to meet primary metabolic needs while addressing economic and environmental sustainability criteria. In this study, phosphorus was consumed at a considerably lower rate compared to nitrogen. This phenomenon suggests a likely abundance of phosphorus in the standard cultivation medium, making reintroduction unnecessary, at least in the first or the first few water reuse cycles. Also, the results indicated that micronutrient consumption may be reduced by up to 77 % across three water reuse cycles. Additionally, this research addresses the environmental challenge posed by the addition of sulphuric acid to obtain low pH conditions in the cultivation medium. By eliminating the need to adjust the pH, the overall volume of acidic wastewater requiring treatment would be reduced, as well as the need for chemicals. This achievement would have broader implications for sustainable and environmentally friendly microalgal cultivation practices.

To ensure high biomass quality in successive cultivation cycles conducted in reused water, the final *C-PC* accumulation in *G. sulphuraria* achievable within the flat panel PBR was finally evaluated. At the end of the cultivation period, the *C-PC* accumulated was found to be the 10.80 ± 0.36 % *w*/w in the control conditions (Table 2). This value is between the highest ever reported for several *G. sulphuraria* strains grown with different trophic modes (Abiusi et al., 2022; Wan et al., 2016; Wang et al., 2020). Nevertheless, it is worth mentioning that the *C-PC* accumulation at the end of a batch cultivation process is maximized, as cultures are dense, and light becomes the limiting factor. Continuous cultivation experiments should be performed to evaluate and maximize the *C-PC* volumetric and areal productivities, appropriately selecting the most appropriate conditions, namely, biomass concentration, dilution factor, and light intensity. As reported in Table 2, the final *C-PC* accumulation did not vary when reusing water as partial cultivation medium for consecutive cycles, or at the maximum recoverable volume, regardless of the harvesting process used. This result implies no major stress factor associated to the strong yellowish color of recovered water, nor the possible presence of *AOM*, over *C-PC* accumulation.

4. Conclusion

The successful reuse of acidic wastewater in *Galdieria sulphuraria* cultivation was discussed in this study. Two harvesting techniques were evaluated and compared to concentrate biomass and extract water for reuse, namely, membrane microfiltration and centrifugation. Results showed that directly reusing 25 % of water did not significantly affect the growth or the quality of *G. sulphuraria* in subsequent cultivation cycles. Moreover, reusing the maximum recoverable freshwater derived from both harvesting processes did not affect the *G. sulphuraria* growth nor the final *C-PC* accumulation, at least for one cycle of reuse. The assessment of more consecutive water reuse cycles is currently under investigation. Appropriate nutrient re-integration was necessary to achieve such goals. Directly reusing highly acidic (pH 2–3) wastewater and micronutrients in *G. sulphuraria* schemes may represent a key step forward in making microalgae cultivation more sustainable by reducing the amount of required freshwater and minimizing the release of acidic wastewater.

Size and energy analyses performed in this work suggest that centrifugation may be more appropriate for small to medium-size biomass cultivation applications, whereby small volumes of biomass and/or high algal cell concentrations in the final biomass product are involved. On the other hand, the adoption of microfiltration units, possibly as pre-treatment for a final concentration step, would allow reducing the overall energy consumption when large volumes of flow rate of biomass suspensions need to be harvested. The potential of this approach for further optimization and scale-up should be investigated in future studies, with the aim of achieving higher levels of water reuse, metals recycling, and ultimately, a more efficient and environmentally friendly process for large-scale microalgae cultivation.

Table 2
G. sulphuraria C-PC accumulation as % weight/weight at the end of each cultivation cycle ($n = 2$).

Conditions	C-phycocyanin [w/w %]
Control	10.80 ± 0.36
Centrifugation steps	
Cycle 1 25 % water reuse	10.46 ± 0.28
Cycle 2 25 % water reuse	10.12 ± 0.63
Cycle 3 25 % water reuse	10.16 ± 0.84
Max water reuse (98 %)	10.79 ± 0.46
Membrane microfiltration steps	
Cycle 1 25 % water reuse	9.46 ± 0.48
Cycle 2 25 % water reuse	10.71 ± 0.93
Cycle 3 25 % water reuse	9.88 ± 0.66
Max water reuse (71.5 %)	11.09 ± 0.76

CRediT authorship contribution statement

M. Carone: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Malaguti:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **M. Zanetti:** Writing – review & editing, Resources, Funding acquisition. **A. Tiraferri:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **V.A. Riggio:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vincenzo A. Riggio reports financial support was provided by Polytechnic University of Turin. Mariachiara Zanetti reports financial support was provided by Polytechnic University of Turin. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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