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# Microalgae Biomass Concentration and Reuse of Water as New Cultivation Medium using Ceramic Membrane Filtration

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## Abstract

The aim of this study is to advance means for microalgae concentration with the simultaneous reuse of water as new cultivation medium, specifically, through ceramic membrane filtration. Three algae, namely, *Spirulina platensis*, *Scenedesmus obliquus*, and *Chlorella sorokiniana* were tested by filtering suspensions with four ceramic membranes having nominal pore sizes of 0.8 μm, 0.14 μm, 300 kDa, 15 kDa. The observed flux values and organic matter removal rates were directly related to the membrane pore size, with some differences in productivity between algae types, likely due to cell size and shape. Interestingly, similar near steady-state fluxes (70-120 L $m^{-2}h^{-1}$) were measured using membranes with nominal pore size above 15 kDa, suggesting the dominance of cake layer filtration independently from the initial flux. Virtually complete algae cells rejections and high nitrate passage (>75%) were observed in all combinations. When the permeate streams were used as media for new growth cycles of the various algae, no or little growth was observed with *Spirulina p.*, while *Chlorella s.* (permeate from 300 kDa membrane) and especially *Scenedesmus o.* (permeate from 0.14 μm membrane) showed the fastest growth rates, not too dissimilar to those observed with ideal fresh media.



**Keywords:** Microfiltration; microalgae harvesting; water reuse; zero-liquid discharge; algae cultivation.

# 1 Introduction

Microalgae market is experiencing a significant growth in a plethora of commercial applications. Among the possible uses of microalgae, water and wastewater treatment, cosmetics, food and biotechnology already represent mature sectors (Kusmayadi et al., 2021; Miguel et al., 2021). More recently, important achievements have been achieved also in the use of algae for $CO_2$ fixation and biofuel production (Goh et al., 2019; Mathimani and Mallick, 2018). Indeed, biofuel production from microalgae is currently considerably more efficient if compared to traditional feedstocks, such as corn and other crops (Goh et al., 2019). Microalgae represent also a valuable asset for the treatment of concentrated industrial flue gases, since promising $CO_2$ fixation rates of roughly 5 $gCO_2\ L^{-1}day^{-1}$ were observed in ambient air, highlighting higher efficiencies than terrestrial plants (Lim et al., 2021).. Nevertheless, the harvesting process of microalgae consumes large water volumes and it is associated with high energy absorption and relevant cost investments, which combined are estimated to account for over the 30% of the total production costs (Wu et al., 2018). In fact, large-scale cultivations of microalgae are rarely feasible as of today.

Technology advances are mandatory to allow the recycling of the harvesting water, thus reducing water and nutrients usage up to percentage values of 84% and 55%, respectively, according to literature reports (Yang et al., 2011). From this perspective, an optimization of the harvesting techniques that also includes water reuse is important for achieving sustainable solutions in terms of both energy and water consumption (Bamba et al., 2021; Fret et al., 2020; Li et al., 2020). A recent review on water recycling in microalgae cultivation has highlighted limitations and potential benefits of this strategy, strongly suggesting that the success of further cultivation in reused water is varied and may depend on various factors, mainly, the algae strain and the quality of the recycled stream (Farooq et al., 2015). Another review work indicated that accumulated ions, dissolved organic matter, residual flocculants,

69 and cell debris can negatively affect the water reusability (Lu et al., 2020). Clearly, the
70 characteristics of the reused water strongly depend on the separation process deployed for
71 algae harvesting and concentration.
72 The most applied harvesting methods to concentrate algae biomass are: (i)
73 coagulation/flocculation, (ii) dissolved air flotation, (iii) electrically run processes, (iv)
74 centrifugation, and (v) membrane filtration (Ferreira et al., 2020; Singh and Patidar, 2018).
75 Membrane-driven separation processes have advantages in terms of footprint and
76 effectiveness, allowing also the concentration of high quality biomass (Singh and Patidar,
77 2018; Zhang and Fu, 2018). In fact, it has been observed that algal cells are less prone to
78 damage during the filtration and thus their reproduction capacity should not be affected
79 (Petruševski et al., 1995). Specifically, microfiltration (MF) and ultrafiltration (UF) are the
80 most promising pressure-driven membrane separation techniques for this specific application:
81 these can harvest algal biomass achieving high concentration factors while using low
82 operating pressures (Ahmad et al., 2012).
83 When it comes to the reuse of permeate streams upon algae concentration achieved with
84 membrane-based processes, sparse and dissimilar results have been reported. For example,
85 *Scenedesmus acuminatus* growth in a water stream obtained upon filtration of the algae
86 suspension with a 50 kDa cut-off PVDF membrane was found to be strongly inhibited by
87 organic matter, with a 13.4% rate of growth observed with respect to fresh media (Lu et al.,
88 2019). On the other hand, Nędzarek et al. evaluated the composition of the permeate obtained
89 by filtering *Monoraphidium contortum* through a 300 kDa UF membrane, specifically
90 focusing on the presence of macronutrients necessary for new cultivation cycles, and
91 indicated that high concentrations phosphorus and nitrogen imply high potential for reuse
92 (Nędzarek et al., 2015). The varied reports available in the literature underline the current
93 incomplete knowledge in this field and imply that further efforts are required to identify the

94 combinations of algae, membranes, and conditions that would result in successful water reuse
95 (Discart et al., 2014; Farooq et al., 2015; Fret et al., 2020; Hwang and Rittmann, 2017; Loftus
96 and Johnson, 2019; Lu et al., 2020; Lu et al., 2019; Nędzarek et al., 2015; Sha et al., 2019;
97 Wang et al., 2018; Zhang et al., 2010; Zhang et al., 2016).
98 Another factor that greatly influences both the performance of membrane-based microalgae
99 concentration and permeate stream reusability is fouling (Discart et al., 2014). It has been
100 highlighted how fouling phenomena are particularly detrimental when membranes are used
101 for algae concentrations (Bamba et al., 2021; Novoa et al., 2021). In this respect, ceramic
102 membranes have been suggested as effective solutions for algae dewatering due to their
103 chemical resistance under a wide range of cleaning conditions. This characteristic allows
104 substantial values of flux recovery during the cleaning processes, as well as to allow
105 sterilization of the systems when they needs to be deployed with different strains or after
106 intense biological contamination (Ahmad et al., 2013; Wu et al., 2018). Reports indicate that
107 membrane pore size greatly influences both fouling behavior and system performance, and
108 that the selection of the appropriate porosity and operating conditions can successfully reduce
109 the detrimental effects of fouling and promote water reuse (Zhang and Fu, 2018; Zhao et al.,
110 2017).
111 In this research, we discuss how MF/UF may be applied to concentrate algae biomass and
112 simultaneously reuse the water as new medium for other cycles of microalgae cultivation.
113 Specifically, suspensions of three algae strains (*Spirulina platensis*, *Scenedesmus obliquus*,
114 *Chlorella sorokiniana*) are filtered through ceramic membranes with different pore sizes to
115 find the best combinations that would maximize productivity and permeate reuse potential .
116 Flux behavior is presented as a function of time and recovery rate, together with removal
117 rates of nutrients, algal cells, and algal organic matter. The algal cells are re-inoculated in the
118 permeate solutions and the growth rates are compared to those obtained with ideal fresh

media. Finally, the most suitable algae-membrane combinations are proposed, while issues limiting the reuse of water are underlined.

# 2 Materials and Methods

## 2.1 Microalgae cultivation in photobioreactor

The concentration and the reuse of water from the cultivation of three microalgae strains were investigated, namely, *Spirulina platensis* (NIVA-CYA 428), *Scenedesmus obliquus* (SAG 276-3b), and *Chlorella sorokiniana* (NIVA CHL-176) (Franchino et al., 2013). *Spirulina* has a diameter of about 8 μm with an elongated shape, while *Scenedesmus* and *Chlorella* are characterized by a more spherical shape with a diameter of roughly 10 μm and 2 μm, respectively. The biomass was obtained from a medium-size non-commercial photobioreactor, which provided a suspension with microalgae concentration of roughly 1 g/L. The biomass concentration was estimated from dry weight measurements, conducted by first filtering the suspension through glass microfiber filters with pore size equal to 1.5 μm and then using a thermal scale operating at 120 °C for 10 min to eliminate the remaining moisture. The optimal growth medium used for algae cultivation was the BG-11 medium (pH 7) for *Scenedesmus o.* and *Chlorella s.*, and the AO medium (pH 9.5) for *Spirulina p.*; see Supplementary Data file for the composition of the two media. The growth rates in the BG-11 and AO media were used as benchmark to compare the growth rates obtained using the reused water collected from the membrane filtration system (permeate water).

## 2.2 Microfiltration concentration process and materials

The microfiltration tests were performed in a cross-flow lab-scale system. The unit comprises an inverter-controlled volumetric pump (Nuert, Pordenone, Italy), a thermally insulated feed tank, and a tubular membrane housing cell (TAMI Industries, Montreal, Canada) consisting

of a stainless-steel cylinder of 250 mm length and 10 mm inner diameter. A volume of 3 L of initial feed suspension was used for filtration tests, each with duration of three hours. For each algae species, the initial feed concentration was 1 g/L. The cross-flow velocity (CFV) and the trans-membrane pressure (TMP) were set equal to 2 m/s and 1 bar, respectively, adjusted using the pump inverter and a back-pressure valve. The temperature of the system was kept at 20±2 °C during the entire filtration. The permeate flux was calculated continuously by monitoring the change in volume over time of the increasing permeate stream collected in a small tank placed on a computer-interfaced balance, with weight measurements taken every 3 min. Four $TiO_2$-based ceramic membranes purchased from TAMI Industries (Montreal, Canada) were selected to evaluate the membrane filtration. Their pore sizes were 0.8 μm, 0.14 μm, 300 kDa, and 15 kDa. Each tubular membrane had an inner diameter of 6 mm, 250 mm length, and an active filtration area of 47.1 $cm^2$. Each of the three algae strain was cultivated in the photobioreactor and then the suspension filtered through each of the four membranes, for a total of 12 combinations. Filtration tests were conducted in duplicates.

Two fouling indices were applied to quantitatively evaluate the membranes performance in terms of flux loss over time and flux recovery after cleaning. Specifically, the flux recovery ratio, defined as $FRR = J_{w2}/J_{w1}$, and the total flux decline ratio, defined as $DRt = 1-(J_p/J_{w1})$ (Ricceri et al., 2021). $J_{w1}$ and $J_p$ represent the pure water flux measured with the pristine membrane and the stabilized flux at the end of the 3-hour filtration process, respectively, while $J_{w2}$ is the flux measured with pure water after the cleaning procedure described in the following. After filtration of the biomass, chemical and physical cleaning was applied to recover the membrane flux. The cleaning was performed in three steps: (i) three quick tap water flushing steps of the feed loop to replace the algae culture in the filtration unit; (ii) two physical cleaning steps with demineralized water flowed in the feed loop for at least 10 min

each; (iii) backwash at 2 bar of pressure using a cleaning solution composed by 6 mL/L of NaClO and 1.5 g/L of citric acid and a temperature of ~65-70 °C. These conditions were chosen to remove and inactivate all the microalgae in the system and allow for the utilization of the filtration unit in a new filtration test with the same or with different algae strains.

### 2.3 **Rejection measurements**

The removals of algae organic matter (AOM) and nutrients by the membranes were evaluated for each filtration test. The overall observed rejection, R(%), is calculated as $1-(c_{permeate}/c_{feed})$, where $c_{permeate}$ and $c_{feed}$ indicate the concentration of a component in the total permeate volume collected at the end of each test and in the concentrated feed obtained at the end of each test, respectively. $NO_3$ was used as representative molecule for the behavior of nutrients, since nitrogen is the most abundant one in the cultivation medium, while dissolved organic carbon (DOC) was set as a proxy for AOM concentration. DOC measurements were performed with 40 mL samples with a Shimadzu (Milan, Italian branch) TOC-LCSH FA, E200 (catalytic oxidation on Pt at 680 °C), after filtration through 0.45 μm filters. DOC analyses were performed in non-purgeable organic carbon mode, following appropriate calibration (Haberkorn et al., 2019). Algae cells rejection was also evaluated through cell counting using a fluorescence microscope (Axioscope 5, Zeiss), which allowed a direct quantification of algae cells in the feed, concentrate, and permeate samples.

### 2.4 **Assessment of water reuse potential**

Before algae re-inoculation into the permeate solution, nitrate and phosphate were reintegrated to reach the standard conditions of the relative optimum medium. Ionic concentrations were measured with an Eco IC ion chromatography system purchased from Metrohm. The growth in these solutions was conducted in 250 mL laboratory flasks and the algae concentration was monitored by means of optical density (OD) measurements

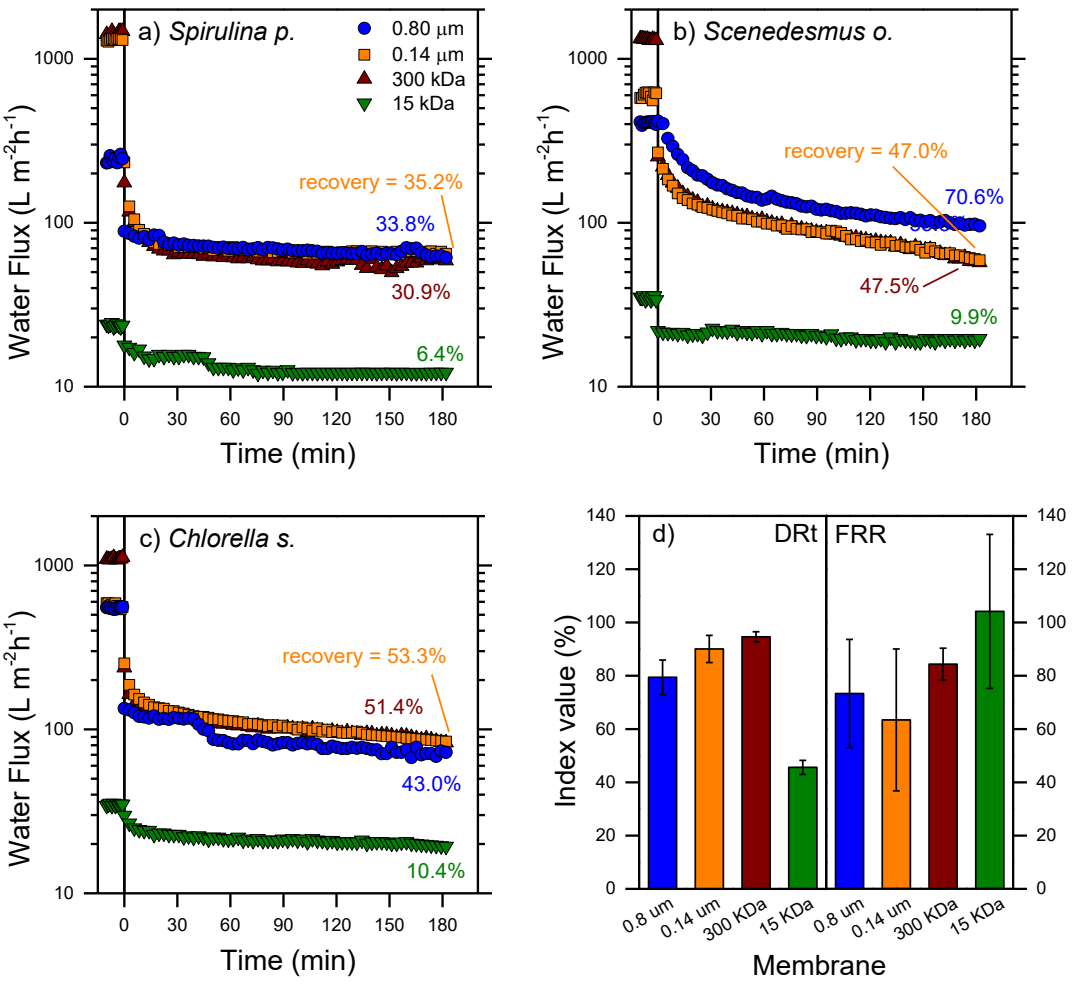
conducted with a UV-31 spectrophotometer (Onda) at 680 nm wavelength. The initial concentration of microalgae was set to achieve an approximate OD value of 0.5 (~0.2 g/L), to have a reference common starting point. The growth of biomass was thus assessed for the following 10 days. Flask illumination was provided h 24/24 with a neon light (Extrastar T5-13W) and the suspensions were continuously agitated at 100 rpm. Each day, the OD was measured on duplicate samples. The resulting OD increase was fitted with a line, and the fitting was considered reliable only when associated with an $R^2$ higher than 95%. The slopes of the OD fitting lines (dOD/dt) observed with the reused waters collected in the filtration tests performed with the various membranes were compared with that observed when each microalgae strain was grown in its respective ideal medium.

# 3 Results and discussion

## 3.1 Productivity observed for different membrane-algae combinations

The first set of tests had the goal to investigate the productivity of the various membranes in the filtration of microalgae, which would inform the most appropriate pore size to concentrate each of the three strains (Hung and Liu, 2006; Zhu et al., 2022). The average fluxes measured in the filtration tests for all membrane-algae combinations are displayed in Figure 1a-c. The values of final achieved recovery are also reported next to each curve and indicate the amount of extracted permeate with respect to the initial feed volume. As expected, the pure water flux, $J_{w1}$, reported before time 0, was proportional with membrane pore sizes, with values higher than 200 L $m^{-2}h^{-1}$ for membranes with pore size ≥ 300 kDa and roughly 30 L $m^{-2}h^{-1}$ for the smaller pore dimensions of 15 kDa. When pure water was replaced by the algae feed (time zero), the permeate flux ($J_p$) showed a very rapid reduction, typically in the first 20 min of filtration. This sudden flux decline was likely due to the development of a microalgae-rich cake layer. This trend in coherent with what observed in other literature studies (Hung and

215 Liu, 2006; Zhang et al., 2010). Following this phase, the flux reached a near-stable value or
216 slowly declined as the feed suspension became increasingly concentrated with time.
217 Differently from $J_{w1}$, the value of $J_p$ did not correlate with the nominal pore size. The
218 elongated filamentous shape of *Spirulina p.* might be responsible for the more rapid flux
219 decline and lowest long-term flux value. On the other hand, spherical shaped *Scenedesmus o.*
220 and *Chlorella s.* showed a smoother flux decline. *Chlorella s.* cells have smaller average
221 diameter than *Scenedesmus o.* cells, possibly penetrating inside the membrane pores causing
222 more severe fouling (Liao et al., 2018; Novoa et al., 2021). Overall, the three more porous
223 membranes displayed similar productivity in the long-term, with fluxes in the range 70-100 L
224 $m^{-2}h^{-1}$ when using different algae. Above 15 kDa, the resistance to flux appeared to be
225 dominated by the algae cake layer: note that nominal pore size of the 300 kDa membrane is
226 roughly 10 times smaller than the size of the most porous membrane (0.8 μm). On the other
227 hand, the final flux measured with the membranes characterized by the narrowest pores
228 tended to always reach a much lower value of approximately 10 L $m^{-2}h^{-1}$, regardless of the
229 algae strain. These results are in good agreement with previous research reporting that similar
230 fluxes, once stabilized, were achieved with both MF and UF membranes (Sun et al., 2013).
231 This effect is also due to the fact that permeate flux in MF membranes is initially higher
232 compared to UF, with a consequent higher fouling tendency.
233 The comparable values of final water flux for the three more porous membranes suggest that
234 a dynamic cake layer, called dynamic membrane, represented the dominant resistance and
235 governed the process above a certain nominal membrane porosity (Bilad et al., 2014; Ersahin
236 et al., 2012; Nguyen et al., 2012). These observations are coherent with previous ones that
237 suggested that pure water flux has little correlation with the value of possible critical flux in
238 MF or UF of colloidal suspensions (Bacchin et al., 2006; Gésan-Guiziou et al., 2002).




239 **Fig. 1**. Productivity measured for different algae-membrane combinations. Water flux
240 observed with different membranes as a function of time with: a) *Spirulina p.*; b)
241 *Scenedesmus o.*, and c) *Chlorella s.* The final value of recovery (collected permeate volume
242 divided by initial feed volume) is reported next to each profile. d) Fouling indices evaluated
243 for each algae-membrane combination.

244

245 As previously mentioned, according to our results, different cells shapes and physical
246 properties may affect the development and the characteristics of the cake layer, such as its
247 porosity and compactness, which in turn yield different resistances to filtration. Such
248 behavior was observed in previous research, whereby different cake layer resistance build-up
249 was observed during the filtration of various microalgae. Specifically, the cake layer formed

by *Chlorella vulgaris* had lower resistance than that associated with *Chlamydomonas reinhardtii*, with this result attributed to the different flexibility of the cells walls of the two algae strains (Marbelia et al., 2016; Shekhar et al., 2017)

Figure 1d summarizes the productivity results by means of two indices, the total flux decline ratio (DRt) and flux recovery ratio (FRR). The values reported in the graph are calculated by averaging the behavior obtained with all algae species for each membrane pore size. DRt slightly increased with smaller pore size, mirroring the lower flux loss upon dynamic membrane formation relative to pure water flux, but was low for the membrane with pore size 15 kDa. A value of 0.5 for the latter membrane means that half of the productivity was lost when pure water was substituted with algae suspensions in the feed. FRR values ranged from 60 to 100%, indicating high efficiency of the cleaning protocol to recover nominal productivity. This result is in good agreement with the study by Wu *et al*. (Wu et al., 2018), who reported a promising and steady filtration performance with low amount of irreversible fouling under conditions similar to those investigated in this study. Specifically, in this study the cleaning performance generally increased as the pore size decreased, indirectly suggesting that fouling mechanisms were progressively more detrimental for smaller pores. This result is consistent with previous reports (Silalahi and Leiknes, 2009). For example, pore blocking phenomena are usually more pronounced in MF/UF applications as the foulant size to pore size ratio is close to 1: in this case, AOM and in general extracellular components may have blocked smaller pores more effectively. This rationalization may also partly explain the large standard deviation calculated for the two membranes with larger pore size, for which instead algae cell themselves may function as pore blocking agents.

Finally, further implications of the filtration results may be highlighted by assessing the data summarized in Figure 2, which reports the values of the measured water fluxes at selected recovery values. This representation helps performing a more robust and rigorous comparison

of the productivity in the various tests: as also observed by other researchers (Zhang et al., 2010), the flux is also affected by feed suspension concentration, which in our tests changed in time and did not reach the same final value for all membrane-algae combinations. The results indicate that, when considering productivity as the target parameter for maximization, *Spirulina p.* may be concentrated with membranes with any pore size equal or above 300 kDa, *Scenedesmus o.* with membranes of large (0.8 µm) pore size, while *Chlorella s.* with medium-sized pores. Algae shape and size may be the main characteristics affecting this result. However, when it comes to coupling effective biomass concentration with potential permeate water reuse, the quality of the permeate stream plays a role at least as important as productivity. The selectivity of the different membranes is discussed below and provides additional complexity to the system and to analyses aimed at the selection of the most appropriate membrane for each algae strain with the goal of water reuse.

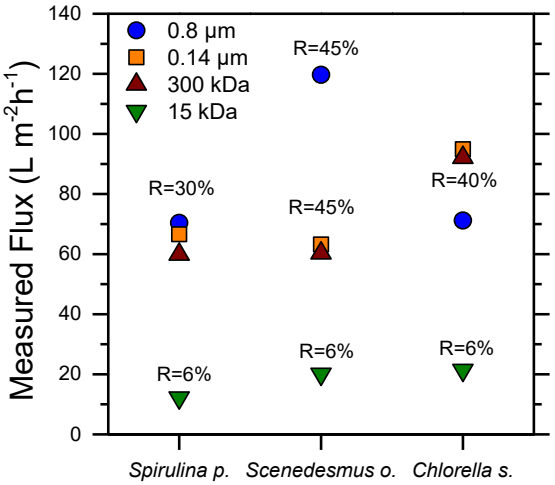



**Fig. 2**. Comparison of the measured fluxes obtained with algae-membrane combinations at various values of recovery rate values.

### 3.2 Separation of algae cells, organic matter, nutrients

To be able to reuse the permeate stream effectively as a medium for subsequent cycles of algae growth, the filtration process should ideally retain all unwanted matter in the

293 concentrate (beside the biomass that needs to be concentrated), namely, accumulated ions,
294 cell debris, and dissolved organic matter, while allowing the passage of all the beneficial
295 components (Discart et al., 2014; Lu et al., 2019). In this case, algal cells and AOM should be
296 retained, while macro and micro-nutrients should be found in the permeate stream for their
297 reuse. Algae cells removal was semi-quantitatively evaluated observing samples of the initial
298 feed, the concentrate, and the permeate samples under a microscope. Fig. 3 shows that the
299 algal cells removal efficiency was virtually complete (~100%): the concentrate suspensions
300 (column b) showed a considerable higher density of algae if compared to the respective initial
301 feed (column a), while the permeate solutions (column c) did not contain appreciable
302 amounts of algal biomass. Microscope images of initial feed, concentrate, and permeate,
303 taken in both normal and fluorescence mode for each strain, are reported in the
304 Supplementary Data.
305
306

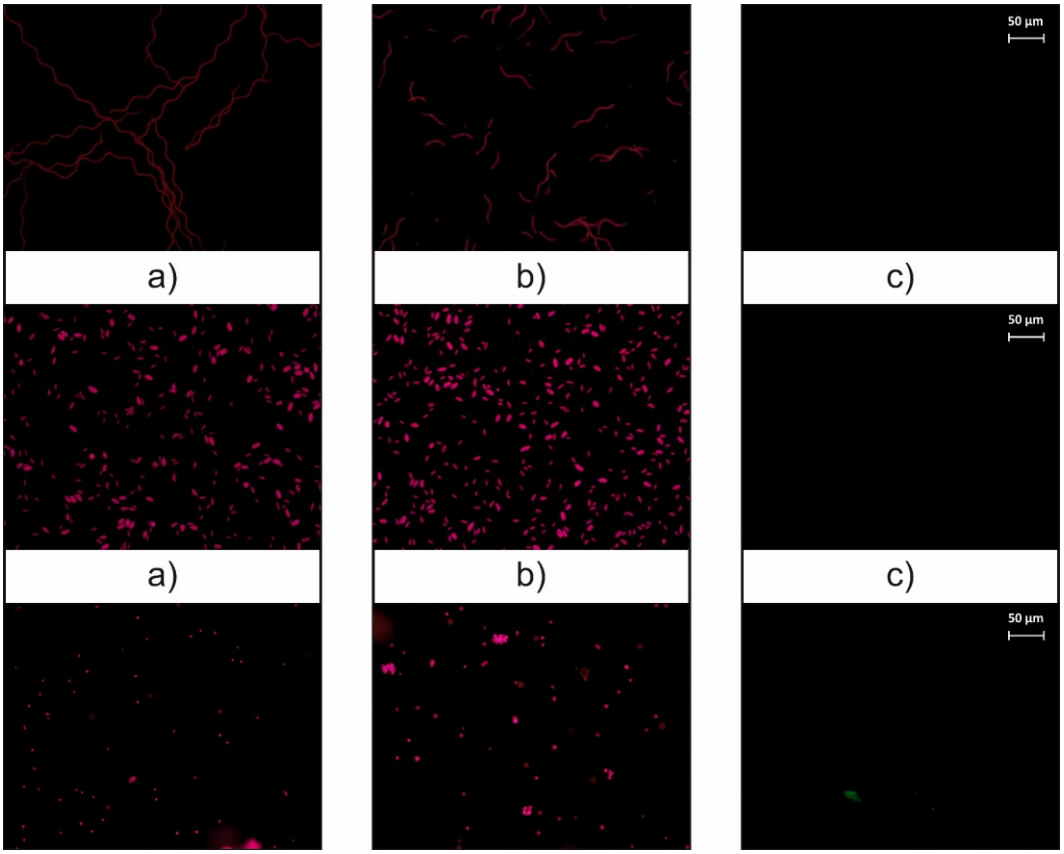



**Fig. 3.** Representative fluorescence microscope images of samples obtained filtering with 300 kDa membrane the feed streams of: (first row) *Spirulina p.*; (second row) *Scenedesmus o.*; (third row) *Chlorella s*. The columns display images for: (a, first column) the initial feed algae suspension; (b, second column) the final concentrate suspension obtained at the end of the filtration process; (c, third column) the final collected permeate. Red and green cells in the photos refer to alive and dead algae, respectively.

Fig. 4 summarizes the results obtained in terms of AOM and nitrate rejections. Results were not reported for *Spirulina p.*, as both nutrients and DOC levels were below instrument's detection limits in the permeate stream. For both *Scenedesmus o.* and *Chlorella s*., a strong correlation can be observed between the DOC rejection and membrane nominal selectivity: higher rejection values were measured when the pore size was smaller. This trend is

consistent with the study by Luo *et al*. (Luo et al., 2019), who analyzed the relation between molecular weight (MW) of organics and their rejection in microfiltration, finding that rejection increased when the organic to membrane pore size ratio increased. The same trend was also reported by Villacorte *et al.*, who observed that the rejection of both AOM and biopolymers increased when lowering the membranes pore size in MF/UF processes (Villacorte et al., 2015). In our study, ultrafiltration membranes ranging from 300 to 15 kDa were likely able to remove medium to low molecular weight compounds (Zhang et al., 2013). Note that the DOC rejection values for *Scenedesmus o.* suspensions were significantly lower than those observed with *Chlorella s*. This result may be due to diverse types and size of AOM and other algal debris produced by the two strains.

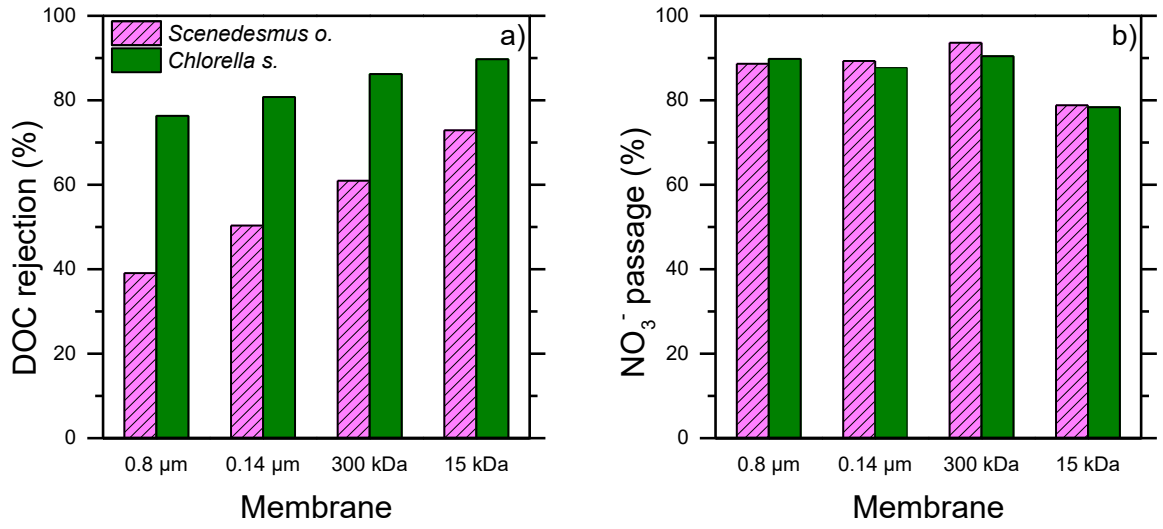



**Fig. 4**. Separation performance of different membranes in terms of a) DOC rejection; b) $NO_3^-$ passage with suspensions containing (patterned pink) *Scenedesmus o.* and (solid green) *Chlorella s.* microalgae. In a), the DOC rejection is used as a proxy for AOM removal. The behavior of *Spirulina p.* is not reported since nitrate and AOM amounts were always under the detection levels in the permeate stream.

On the other hand, Fig. 4b shows that the passage of $NO_3^-$ into the permeate stream was generally relevant and similar (~88-93%) when the membrane pore size was above 15 kDa. High passage implies lesser need of salts reintegration before the subsequent cultivation process, with a resultant positive effect in term of both economics and sustainability of the process. Indeed, nitrates and other ions are significantly smaller than the pore size of the investigated membranes and high passage was expected. That being said, the data suggests that a fraction of nitrate was rejected, probably due to interactions with the cake layer. Note that high nutrients passage is not necessarily desired and balance between nutrients should be the target, instead. Accumulation of non-limiting nutrients or a change in medium salinity may limit new growth cycles (Alyabyev et al., 2007; Rodolfi et al., 2003).

### 3.3 Potential of water reuse for new cultivation cycles

The permeate solutions collected during each filtration experiment were used as a new cultivation media to grow the respective algae species. The performance was always compared with that obtained with the ideal fresh growth medium (Sha et al., 2019; Zhang et al., 2016). Fig. 5 shows optical density values measured during cultivation as a function of time, to assess the evolution of algae biomass concentration. A trend line is shown only when the fitting of the dataset is characterized by an $R^2$ value higher than 95%. Each data point is the result of duplicate experiments, while the $R^2$ is retrieved by the fitting of the entire data population of OD values (2 per day for 10 days). Inconsistent results were obtained with *Spirulina p.*, as no growth was observed with two of the reused permeate solutions, while a trend of growth in the initial 4-5 days was followed by a decline in optical density for the rest of the test in permeates obtained with membrane characterized by pore sizes of 0.8 μm and 300 kDa, the latter being the only case in which a reasonable $R^2$ was obtained. Even so, the results indicate considerable discrepancy in the growth of *Spirulina p.* between this permeate solution (0.049 1/day) and the ideal fresh medium (AO medium). Previous literature studies

and preliminary results (not shown) suggest that the limited growth may be due to imbalance in the concentration of macro- and micro-nutrients in the reused permeate solution and/or to the presence of toxic low molecular weight trace compounds and cell debris (Rodolfi et al., 2003; Zhang et al., 2016). For this reason, knowledge of the performance of a system and of the composition of a permeate stream is crucial to manage the replenishment of macro- and micro-nutrients in the stream, or its partial dilution with freshwater, a topic that merits sustained additional research efforts also from a biological standpoint.

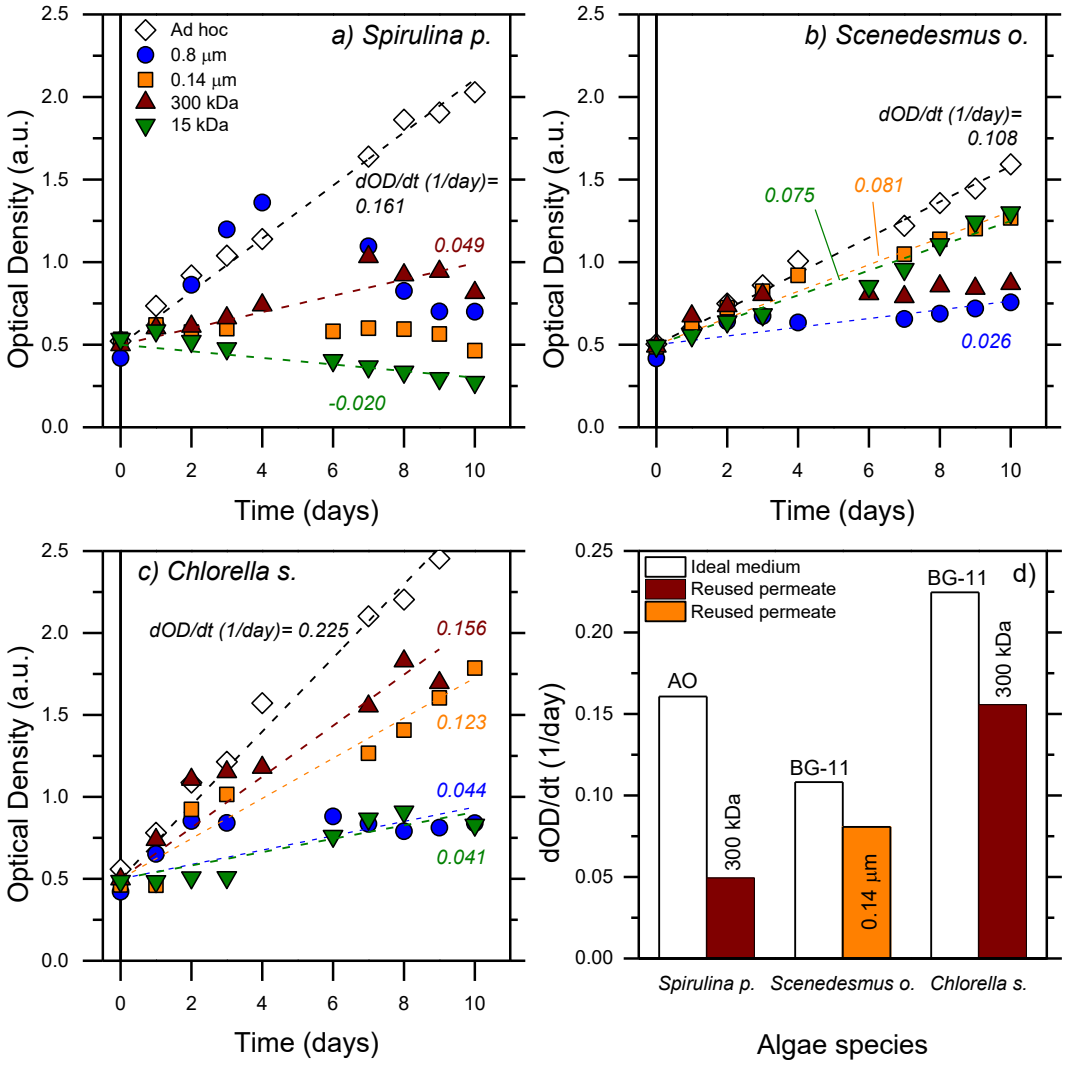



**Fig. 5**. Growth rates in reused water and comparison with fresh ad hoc media for: a) *Spirulina p.*; b) *Scenedesmus o.*; c) *Chlorella s.* Growth rate were estimated trough optical density measurements. Tests were performed in duplicates: data points represent the average value.

371 The dashed lines are the best linear fits of data points and are shown only if the $R^2$ value is
372 higher than 95%. d) Growth rate of the three algae strains in (white bar) each respective fresh
373 ad hoc medium and (colored bars) the reused water showing the best performance among
374 those obtained from filtration tests using membranes of different pore sizes.

375

376 More consistent results were obtained with *Scenedesmus o.* and *Chlorella s.* For *Scenedesmus*
377 *o.*, modest growths were recorded, but quite similar in the reused permeate and in the ideal
378 fresh medium (BG-11 medium). Specifically, 70-75 % of growth rate was estimated in the
379 "15 kDa permeate" and in the "0.14 μm permeate" with respect to the ideal medium (0.108
380 1/day); see Fig. 5b. Given the significant difference in productivity between the two
381 membranes, the membrane with pore size equal to 0.14 μm should be preferred in a potential
382 scale-up. *Chlorella s.* showed the most promising results (Figure 5c). This strain presented
383 the most rapid absolute growth rates. The highest rate (0.156 1/day) was observed in the
384 permeate solution obtained with the 300 kDa membranes, having a value of 69% with respect
385 to the ideal medium (0.224 1/day). Finally, for each algae a summary picture is provided in
386 Fig. 5d, which displays slope values obtained in the ideal medium and with the most suitable
387 reused permeate, selected by taking into consideration both productivity and reuse potential
388 performance. Overall, the growth results obtained in the reused water in this study suggests
389 an intermediate situation between reports that initially discouraged the reuse of algae growth
390 media (Rodolfi et al., 2003) and more recent research suggesting that recycled culture media
391 may be used without any decline in biomass productivity (Fon Sing et al., 2014). Careful
392 control of the cake layer role and of the composition of the reuse streams may be the key for
393 successful reuse of a high percentage of cultivation water.
394 Overall, the results of this research imply that permeate solutions can effectively be reused as
395 new growth media to create a semi-closed harvesting and cultivation cycles. If the permeate

396 is reused without blending and if no further optimization is achieved, the results suggest a
397 maximum retention time of the algae in the photobioreactor equal to 2-3 days during growth
398 cycles. Therefore, it seems appropriate to limit the percentage of reused water by blending,
399 which would imply taking into account discharging a fraction of wastewater and the need for
400 makeup freshwater. However, a large room for improvements exists: for example, the
401 balance of both macronutrients and micronutrients should be carefully assessed, monitored,
402 and managed in the reused water, especially if a substantial portion of water is reused in
403 every cycle, which would be associated with risks of deterioration of the quality of the
404 growth medium after various cycles. Moreover, the presence and toxicity of potential organic
405 by-products in the cultivation step should also be investigated because these compounds may
406 also accumulate in the recycled water.

407 # 4 Conclusions

408 Ceramic MF and UF membranes were evaluated in the harvesting of algal biomass and the
409 permeate stream was reused as new cultivation medium. Ceramic membranes successfully
410 concentrated biomass with productivity of 60-120 L $m^{-2}h^{-1}$ when feed algal concentrations
411 were between 1.6 and 2 g/L. Algae cells were rejected nearly at 100% rate. Instead,
412 membranes retained between 40 and 90% of the small-size or dissolved organic content.
413 Lastly, nitrate passage was almost complete (80 to 95%). Satisfying growth of algae in the
414 reuse permeates were registered with the combinations: (i) *Scenedesmus o.*-0.14 μm
415 membrane; (ii) *Chlorella s.*-300 kDa membrane.

416 **CRediT authorship contribution statement**

417 **Francesco Ricceri:** Conceptualization, Formal analysis, Investigation, Methodology,
418 Validation, Writing – Original draft. **Marco Malaguti:** Data curation, Formal analysis,

Investigation, Methodology, Visualization, Writing – Original draft. **Clara Derossi:** Data curation, Investigation, Methodology. **Mariachiara Zanetti:** Funding acquisition, Project administration, Resources**. Vincenzo Riggio:** Funding acquisition, Project administration, Resources**. Alberto Tiraferri:** Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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**Conflicts of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online.

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