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

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Abstract

24 The aim of this study is to advance means for microalgae concentration with the simultaneous

25 reuse of water as new cultivation medium, specifically, through ceramic membrane filtration.

26 Three algae, namely, *Spirulina platensis*, *Scenedesmus obliquus*, and *Chlorella sorokiniana*

27 were tested by filtering suspensions with four ceramic membranes having nominal pore sizes

28 of 0.8 μm, 0.14 μm, 300 kDa, 15 kDa. The observed flux values and organic matter removal

29 rates were directly related to the membrane pore size, with some differences in productivity

30 between algae types, likely due to cell size and shape. Interestingly, similar near steady-state

31 fluxes (70-120 L m^{-2}h^{-1}) were measured using membranes with nominal pore size above 15

32 kDa, suggesting the dominance of cake layer filtration independently from the initial flux.

33 Virtually complete algae cells rejections and high nitrate passage (>75%) were observed in

34 all combinations. When the permeate streams were used as media for new growth cycles of

35 the various algae, no or little growth was observed with *Spirulina p.*, while *Chlorella s.*

36 (permeate from 300 kDa membrane) and especially *Scenedesmus o.* (permeate from 0.14 μm

37 membrane) showed the fastest growth rates, not too dissimilar to those observed with ideal

38 fresh media.

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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

119   media. Finally, the most suitable algae-membrane combinations are proposed, while issues

120   limiting the reuse of water are underlined.

121   **2   Materials and Methods**

122   **2.1   Microalgae cultivation in photobioreactor**

123   The concentration and the reuse of water from the cultivation of three microalgae strains were

124   investigated, namely, *Spirulina platensis* (NIVA-CYA 428), *Scenedesmus obliquus* (SAG

125   276-3b), and *Chlorella sorokiniana* (NIVA CHL-176) (Franchino et al., 2013). *Spirulina* has

126   a diameter of about 8 μm with an elongated shape, while *Scenedesmus* and *Chlorella* are

127   characterized by a more spherical shape with a diameter of roughly 10 μm and 2 μm,

128   respectively. The biomass was obtained from a medium-size non-commercial

129   photobioreactor, which provided a suspension with microalgae concentration of roughly 1

130   g/L. The biomass concentration was estimated from dry weight measurements, conducted by

131   first filtering the suspension through glass microfiber filters with pore size equal to 1.5 μm

132   and then using a thermal scale operating at 120 °C for 10 min to eliminate the remaining

133   moisture. The optimal growth medium used for algae cultivation was the BG-11 medium (pH

134   7) for *Scenedesmus o.* and *Chlorella s.*, and the AO medium (pH 9.5) for *Spirulina p.*; see

135   Supplementary Data file for the composition of the two media. The growth rates in the BG-11

136   and AO media were used as benchmark to compare the growth rates obtained using the

137   reused water collected from the membrane filtration system (permeate water).

138   **2.2   Microfiltration concentration process and materials**

139   The microfiltration tests were performed in a cross-flow lab-scale system. The unit comprises

140   an inverter-controlled volumetric pump (Nuert, Pordenone, Italy), a thermally insulated feed

141   tank, and a tubular membrane housing cell (TAMI Industries, Montreal, Canada) consisting

142   of a stainless-steel cylinder of 250 mm length and 10 mm inner diameter. A volume of 3 L of

143   initial feed suspension was used for filtration tests, each with duration of three hours. For

144   each algae species, the initial feed concentration was 1 g/L. The cross-flow velocity (CFV)

145   and the trans-membrane pressure (TMP) were set equal to 2 m/s and 1 bar, respectively,

146   adjusted using the pump inverter and a back-pressure valve. The temperature of the system

147   was kept at 20±2 °C during the entire filtration. The permeate flux was calculated

148   continuously by monitoring the change in volume over time of the increasing permeate

149   stream collected in a small tank placed on a computer-interfaced balance, with weight

150   measurements taken every 3 min. Four TiO_2-based ceramic membranes purchased from

151   TAMI Industries (Montreal, Canada) were selected to evaluate the membrane filtration. Their

152   pore sizes were 0.8 µm, 0.14 µm, 300 kDa, and 15 kDa. Each tubular membrane had an inner

153   diameter of 6 mm, 250 mm length, and an active filtration area of 47.1 cm^2. Each of the three

154   algae strain was cultivated in the photobioreactor and then the suspension filtered through

155   each of the four membranes, for a total of 12 combinations. Filtration tests were conducted in

156   duplicates.

157   Two fouling indices were applied to quantitatively evaluate the membranes performance in

158   terms of flux loss over time and flux recovery after cleaning. Specifically, the flux recovery

159   ratio, defined as FRR = J_{w2}/J_{w1}, and the total flux decline ratio, defined as DRt = $1-(J_p/J_{w1})$

160   (Ricceri et al., 2021). J_{w1} and J_p represent the pure water flux measured with the pristine

161   membrane and the stabilized flux at the end of the 3-hour filtration process, respectively,

162   while J_{w2} is the flux measured with pure water after the cleaning procedure described in the

163   following. After filtration of the biomass, chemical and physical cleaning was applied to

164   recover the membrane flux. The cleaning was performed in three steps: (i) three quick tap

165   water flushing steps of the feed loop to replace the algae culture in the filtration unit; (ii) two

166   physical cleaning steps with demineralized water flowed in the feed loop for at least 10 min

167 each; (iii) backwash at 2 bar of pressure using a cleaning solution composed by 6 mL/L of

168 NaClO and 1.5 g/L of citric acid and a temperature of ~65-70 °C. These conditions were

169 chosen to remove and inactivate all the microalgae in the system and allow for the utilization

170 of the filtration unit in a new filtration test with the same or with different algae strains.

171 ## 2.3 Rejection measurements

172 The removals of algae organic matter (AOM) and nutrients by the membranes were evaluated

173 for each filtration test. The overall observed rejection, R(%), is calculated as $1-(c_{permeate}/c_{feed})$,

174 where $c_{permeate}$ and c_{feed} indicate the concentration of a component in the total permeate

175 volume collected at the end of each test and in the concentrated feed obtained at the end of

176 each test, respectively. NO_3 was used as representative molecule for the behavior of nutrients,

177 since nitrogen is the most abundant one in the cultivation medium, while dissolved organic

178 carbon (DOC) was set as a proxy for AOM concentration. DOC measurements were

179 performed with 40 mL samples with a Shimadzu (Milan, Italian branch) TOC-LCSH FA,

180 E200 (catalytic oxidation on Pt at 680 °C), after filtration through 0.45 μm filters. DOC

181 analyses were performed in non-purgeable organic carbon mode, following appropriate

182 calibration (Haberkorn et al., 2019). Algae cells rejection was also evaluated through cell

183 counting using a fluorescence microscope (Axioscope 5, Zeiss), which allowed a direct

184 quantification of algae cells in the feed, concentrate, and permeate samples.

185 ## 2.4 Assessment of water reuse potential

186 Before algae re-inoculation into the permeate solution, nitrate and phosphate were

187 reintegrated to reach the standard conditions of the relative optimum medium. Ionic

188 concentrations were measured with an Eco IC ion chromatography system purchased from

189 Metrohm. The growth in these solutions was conducted in 250 mL laboratory flasks and the

190 algae concentration was monitored by means of optical density (OD) measurements

191 conducted with a UV-31 spectrophotometer (Onda) at 680 nm wavelength. The initial

192 concentration of microalgae was set to achieve an approximate OD value of 0.5 (~0.2 g/L), to

193 have a reference common starting point. The growth of biomass was thus assessed for the

194 following 10 days. Flask illumination was provided h 24/24 with a neon light (Extrastar T5-

195 13W) and the suspensions were continuously agitated at 100 rpm. Each day, the OD was

196 measured on duplicate samples. The resulting OD increase was fitted with a line, and the

197 fitting was considered reliable only when associated with an R^2 higher than 95%. The slopes

198 of the OD fitting lines (dOD/dt) observed with the reused waters collected in the filtration

199 tests performed with the various membranes were compared with that observed when each

200 microalgae strain was grown in its respective ideal medium.

201 ## 3   Results and discussion

202 ### 3.1   Productivity observed for different membrane-algae combinations

203 The first set of tests had the goal to investigate the productivity of the various membranes in

204 the filtration of microalgae, which would inform the most appropriate pore size to concentrate

205 each of the three strains (Hung and Liu, 2006; Zhu et al., 2022). The average fluxes measured

206 in the filtration tests for all membrane-algae combinations are displayed in Figure 1a-c. The

207 values of final achieved recovery are also reported next to each curve and indicate the amount

208 of extracted permeate with respect to the initial feed volume. As expected, the pure water

209 flux, J_{w1}, reported before time 0, was proportional with membrane pore sizes, with values

210 higher than 200 L m^{-2}h^{-1} for membranes with pore size $\geq$ 300 kDa and roughly 30 L m^{-2}h^{-1}

211 for the smaller pore dimensions of 15 kDa. When pure water was replaced by the algae feed

212 (time zero), the permeate flux (J_p) showed a very rapid reduction, typically in the first 20 min

213 of filtration. This sudden flux decline was likely due to the development of a microalgae-rich

214 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).
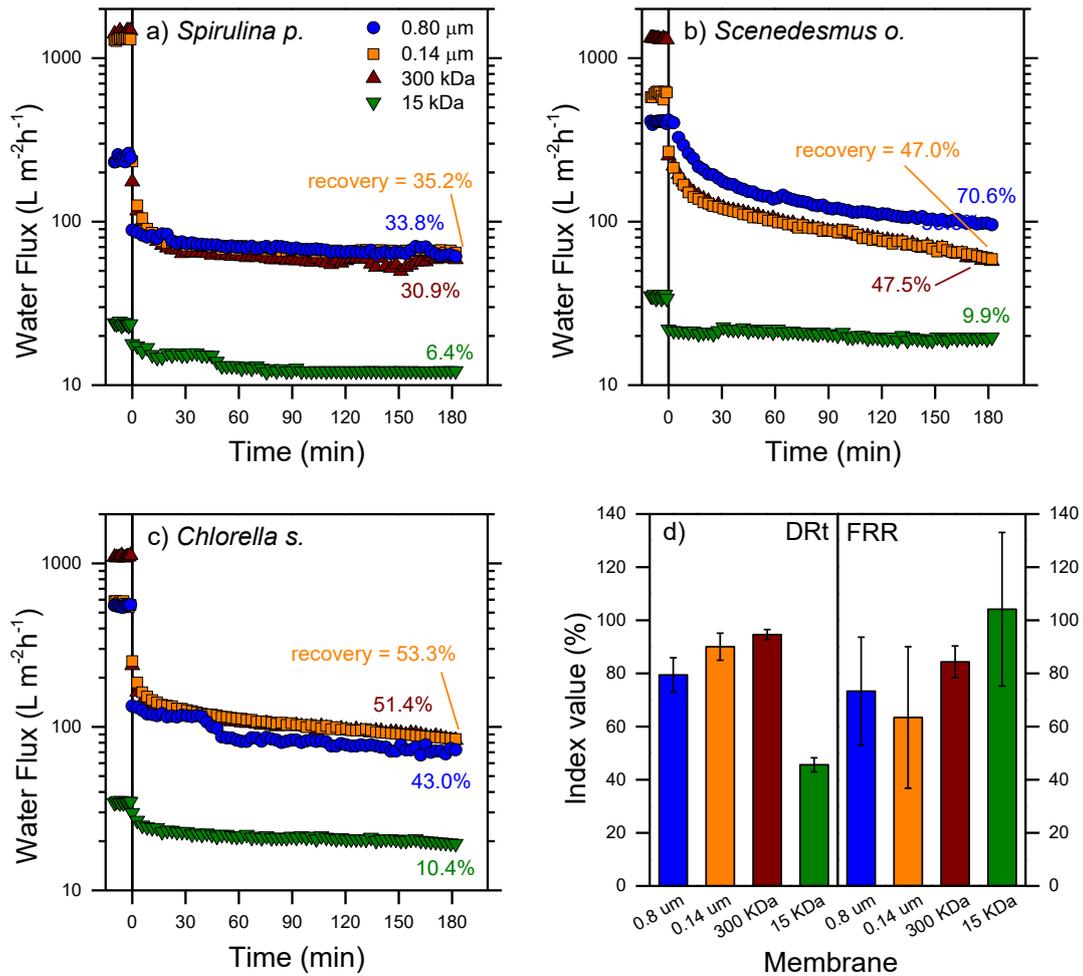
Fig. 1. Productivity measured for different algae-membrane combinations. Water flux observed with different membranes as a function of time with: a) *Spirulina p.*; b) *Scenedesmus o.*, and c) *Chlorella s.* The final value of recovery (collected permeate volume divided by initial feed volume) is reported next to each profile. d) Fouling indices evaluated for each algae-membrane combination.

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

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250   by *Chlorella vulgaris* had lower resistance than that associated with *Chlamydomonas*

251   *reinhardtii*, with this result attributed to the different flexibility of the cells walls of the two

252   algae strains (Marbelia et al., 2016; Shekhar et al., 2017)

253   Figure 1d summarizes the productivity results by means of two indices, the total flux decline

254   ratio (DRt) and flux recovery ratio (FRR). The values reported in the graph are calculated by

255   averaging the behavior obtained with all algae species for each membrane pore size. DRt

256   slightly increased with smaller pore size, mirroring the lower flux loss upon dynamic

257   membrane formation relative to pure water flux, but was low for the membrane with pore size

258   15 kDa. A value of 0.5 for the latter membrane means that half of the productivity was lost

259   when pure water was substituted with algae suspensions in the feed. FRR values ranged from

260   60 to 100%, indicating high efficiency of the cleaning protocol to recover nominal

261   productivity. This result is in good agreement with the study by Wu *et al*. (Wu et al., 2018),

262   who reported a promising and steady filtration performance with low amount of irreversible

263   fouling under conditions similar to those investigated in this study. Specifically, in this study

264   the cleaning performance generally increased as the pore size decreased, indirectly suggesting

265   that fouling mechanisms were progressively more detrimental for smaller pores. This result is

266   consistent with previous reports (Silalahi and Leiknes, 2009). For example, pore blocking

267   phenomena are usually more pronounced in MF/UF applications as the foulant size to pore

268   size ratio is close to 1: in this case, AOM and in general extracellular components may have

269   blocked smaller pores more effectively. This rationalization may also partly explain the large

270   standard deviation calculated for the two membranes with larger pore size, for which instead

271   algae cell themselves may function as pore blocking agents.

272   Finally, further implications of the filtration results may be highlighted by assessing the data

273   summarized in Figure 2, which reports the values of the measured water fluxes at selected

274   recovery values. This representation helps performing a more robust and rigorous comparison

275 of the productivity in the various tests: as also observed by other researchers (Zhang et al.,

276 2010), the flux is also affected by feed suspension concentration, which in our tests changed

277 in time and did not reach the same final value for all membrane-algae combinations. The

278 results indicate that, when considering productivity as the target parameter for maximization,

279 *Spirulina p.* may be concentrated with membranes with any pore size equal or above 300

280 kDa, *Scenedesmus o.* with membranes of large (0.8 µm) pore size, while *Chlorella s.* with

281 medium-sized pores. Algae shape and size may be the main characteristics affecting this

282 result. However, when it comes to coupling effective biomass concentration with potential

283 permeate water reuse, the quality of the permeate stream plays a role at least as important as

284 productivity. The selectivity of the different membranes is discussed below and provides

285 additional complexity to the system and to analyses aimed at the selection of the most

286 appropriate membrane for each algae strain with the goal of water reuse.
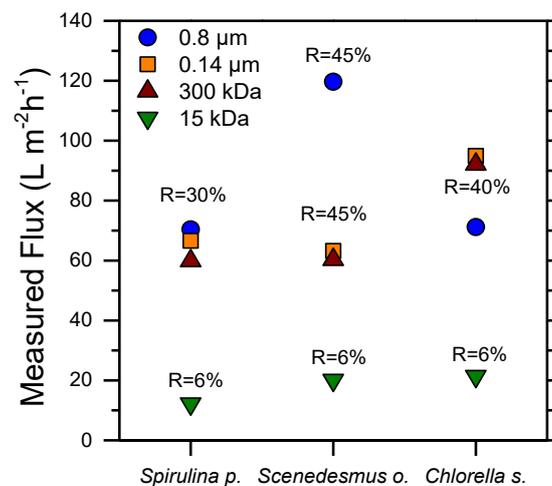
287

288 **Fig. 2**. Comparison of the measured fluxes obtained with algae-membrane combinations at

289 various values of recovery rate values.

290 **3.2   Separation of algae cells, organic matter, nutrients**

291 To be able to reuse the permeate stream effectively as a medium for subsequent cycles of

292 algae growth, the filtration process should ideally retain all unwanted matter in the

13

concentrate (beside the biomass that needs to be concentrated), namely, accumulated ions, cell debris, and dissolved organic matter, while allowing the passage of all the beneficial components (Discart et al., 2014; Lu et al., 2019). In this case, algal cells and AOM should be retained, while macro and micro-nutrients should be found in the permeate stream for their reuse. Algae cells removal was semi-quantitatively evaluated observing samples of the initial feed, the concentrate, and the permeate samples under a microscope. Fig. 3 shows that the algal cells removal efficiency was virtually complete (~100%): the concentrate suspensions (column b) showed a considerable higher density of algae if compared to the respective initial feed (column a), while the permeate solutions (column c) did not contain appreciable amounts of algal biomass. Microscope images of initial feed, concentrate, and permeate, taken in both normal and fluorescence mode for each strain, are reported in the Supplementary Data.
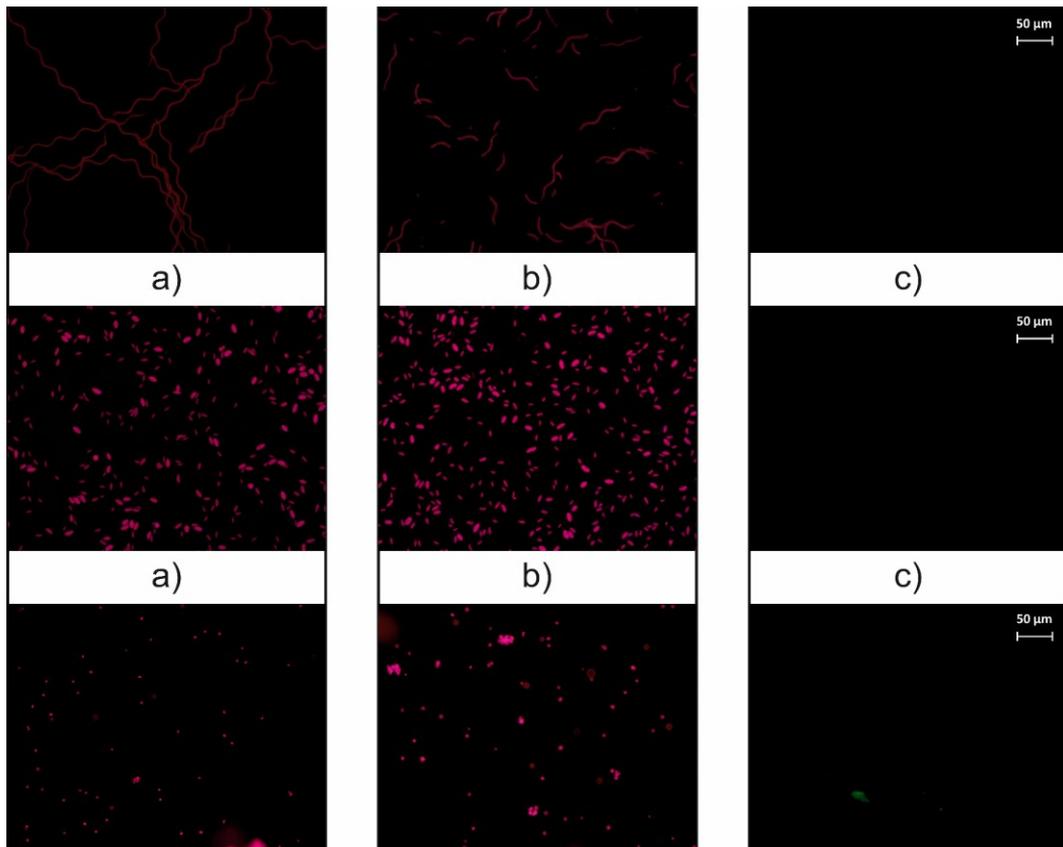
Fig. 3. Representative fluorescence microscope images of samples obtained filtering with 300 kDa membrane the feed streams of: (first row) *Spirulina p.*; (second row) *Scenedesmus o.*; (third row) *Chlorella s*. The columns display images for: (a, first column) the initial feed algae suspension; (b, 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

320 consistent with the study by Luo *et al*. (Luo et al., 2019), who analyzed the relation between

321 molecular weight (MW) of organics and their rejection in microfiltration, finding that

322 rejection increased when the organic to membrane pore size ratio increased. The same trend

323 was also reported by Villacorte *et al.*, who observed that the rejection of both AOM and

324 biopolymers increased when lowering the membranes pore size in MF/UF processes

325 (Villacorte et al., 2015). In our study, ultrafiltration membranes ranging from 300 to 15 kDa

326 were likely able to remove medium to low molecular weight compounds (Zhang et al., 2013).

327 Note that the DOC rejection values for *Scenedesmus o.* suspensions were significantly lower

328 than those observed with *Chlorella s*. This result may be due to diverse types and size of

329 AOM and other algal debris produced by the two strains.
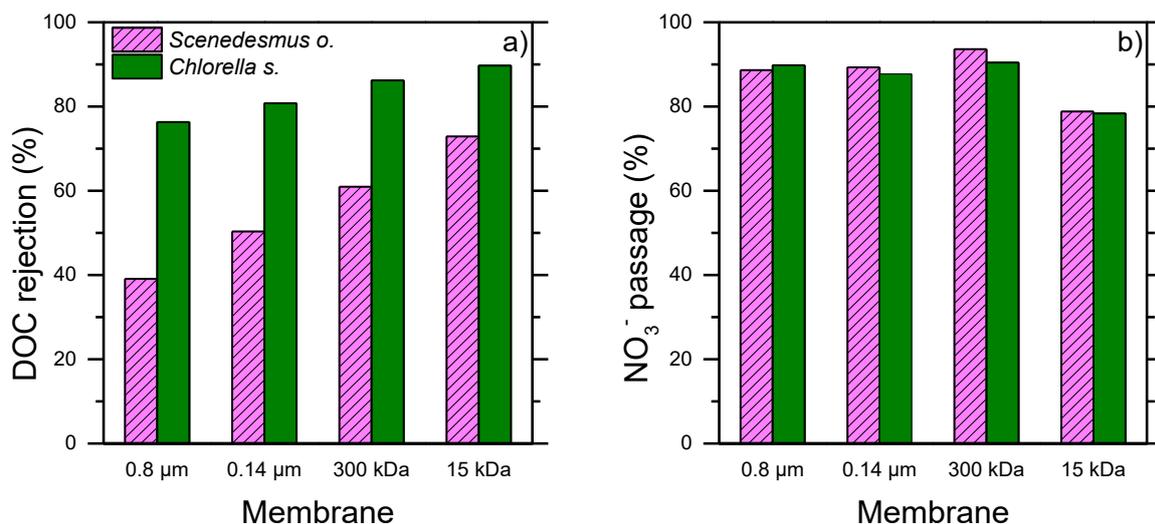
330 **Fig. 4**. Separation performance of different membranes in terms of a) DOC rejection; b) NO_3^-

331 passage with suspensions containing (patterned pink) *Scenedesmus o.* and (solid green)

332 *Chlorella s.* microalgae. In a), the DOC rejection is used as a proxy for AOM removal. The

333 behavior of *Spirulina p.* is not reported since nitrate and AOM amounts were always under

334 the detection levels in the permeate stream.

335

336 On the other hand, Fig. 4b shows that the passage of NO_3^- into the permeate stream was

337 generally relevant and similar (~88-93%) when the membrane pore size was above 15 kDa.

338 High passage implies lesser need of salts reintegration before the subsequent cultivation

339 process, with a resultant positive effect in term of both economics and sustainability of the

340 process. Indeed, nitrates and other ions are significantly smaller than the pore size of the

341 investigated membranes and high passage was expected. That being said, the data suggests

342 that a fraction of nitrate was rejected, probably due to interactions with the cake layer. Note

343 that high nutrients passage is not necessarily desired and balance between nutrients should be

344 the target, instead. Accumulation of non-limiting nutrients or a change in medium salinity

345 may limit new growth cycles (Alyabyev et al., 2007; Rodolfi et al., 2003).

346 **3.3   Potential of water reuse for new cultivation cycles**

347 The permeate solutions collected during each filtration experiment were used as a new

348 cultivation media to grow the respective algae species. The performance was always

349 compared with that obtained with the ideal fresh growth medium (Sha et al., 2019; Zhang et

350 al., 2016). Fig. 5 shows optical density values measured during cultivation as a function of

351 time, to assess the evolution of algae biomass concentration. A trend line is shown only when

352 the fitting of the dataset is characterized by an R^2 value higher than 95%. Each data point is

353 the result of duplicate experiments, while the R^2 is retrieved by the fitting of the entire data

354 population of OD values (2 per day for 10 days). Inconsistent results were obtained with

355 *Spirulina p.*, as no growth was observed with two of the reused permeate solutions, while a

356 trend of growth in the initial 4-5 days was followed by a decline in optical density for the rest

357 of the test in permeates obtained with membrane characterized by pore sizes of 0.8 μm and

358 300 kDa, the latter being the only case in which a reasonable R^2 was obtained. Even so, the

359 results indicate considerable discrepancy in the growth of *Spirulina p.* between this permeate

360 solution (0.049 1/day) and the ideal fresh medium (AO medium). Previous literature studies

361 and preliminary results (not shown) suggest that the limited growth may be due to imbalance

362 in the concentration of macro- and micro-nutrients in the reused permeate solution and/or to

363 the presence of toxic low molecular weight trace compounds and cell debris (Rodolfi et al.,

364 2003; Zhang et al., 2016). For this reason, knowledge of the performance of a system and of

365 the composition of a permeate stream is crucial to manage the replenishment of macro- and

366 micro-nutrients in the stream, or its partial dilution with freshwater, a topic that merits

367 sustained additional research efforts also from a biological standpoint.
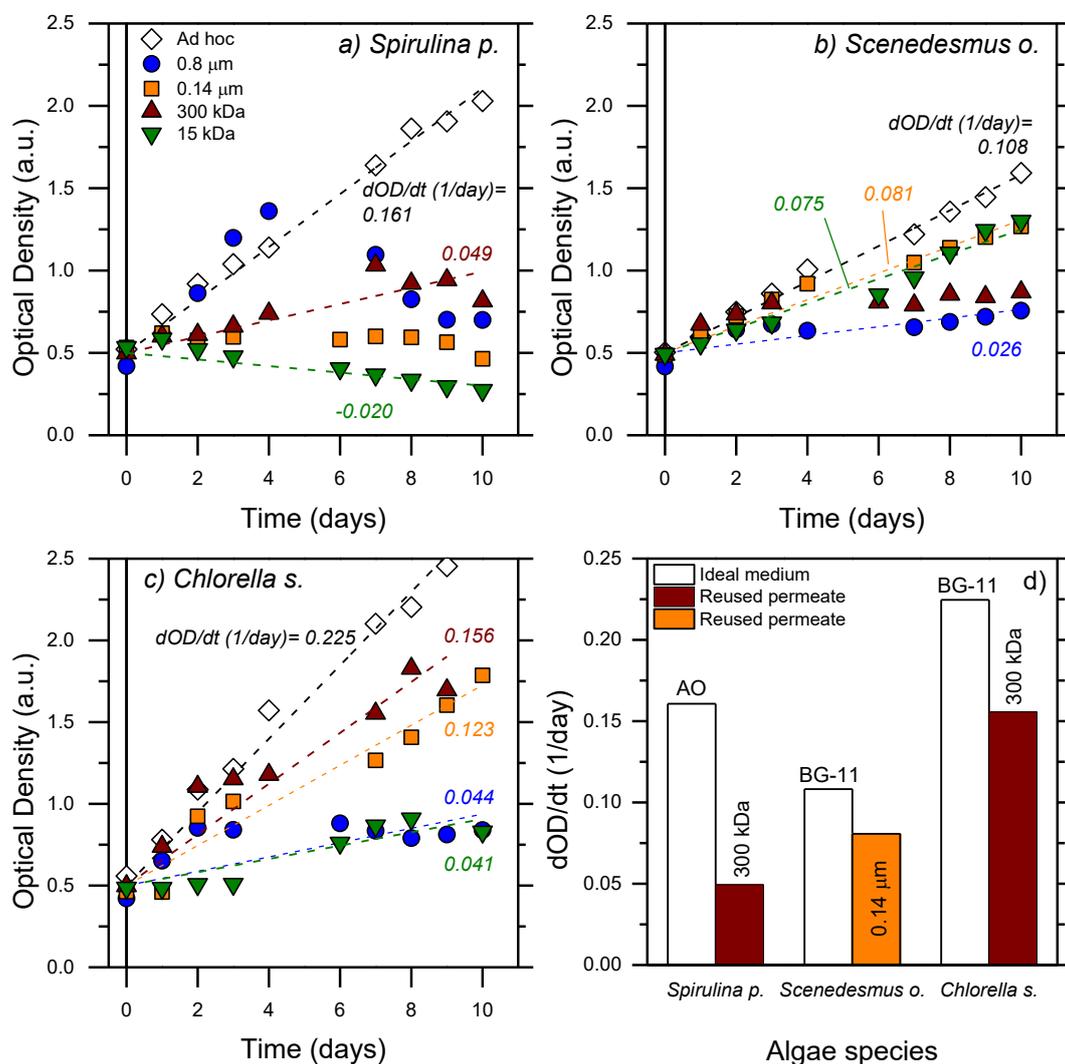
368 **Fig. 5**. Growth rates in reused water and comparison with fresh ad hoc media for: a) *Spirulina*

369 *p.*; b) *Scenedesmus o.*; c) *Chlorella s.* Growth rate were estimated trough optical density

370 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,

419 Investigation, Methodology, Visualization, Writing – Original draft. **Clara Derossi:** Data

420 curation, Investigation, Methodology. **Mariachiara Zanetti:** Funding acquisition, Project

421 administration, Resources**. Vincenzo Riggio:** Funding acquisition, Project administration,

422 Resources**. Alberto Tiraferri:** Funding acquisition, Project administration, Resources,

423 Supervision, Visualization, Writing – review & editing.

427 **Conflicts of interest**

428 The authors declare that they have no known competing financial interests or personal

429 relationships that could have appeared to influence the work reported in this paper.

430 **Appendix A. Supplementary data**

431 Supplementary data to this article can be found online.

432

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