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1 **Separation of lipids and proteins from clarified microalgae lysate: The**
2 **effect of lipid-protein interaction on the cross-flow and shear-enhanced**
3 **microfiltration performances**

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19 **Abstract**

20 For microalgae biorefinery, membrane process is a suitable technology for biomolecules
21 (i.e., proteins, lipids or carbohydrates) fractionation due to the simplicity in operation, the
22 flexibility for implementation and the potential for processing large volumes. Here the objective
23 was to microfiltrate microalgae aqueous extracts, after cell disruption and clarification, to
24 concentrate lipids in retentate and recover hydrophilic proteins in permeate. However, the
25 optimization of the membrane process requires a large amount of raw material and the
26 composition of real microalgae extracts is so complex that it limits the in-depth understanding
27 of the membrane / molecules interactions on the modification of the filtration performances.
28 Thus, simplified model mixtures of clarified microalgae lysates were formulated, aiming at
29 quite a high complexity with constant composition but a nice representativity with respect to
30 the real mixture. Through the microfiltration of three model mixtures containing lipids, proteins
31 or both kinds of molecules, it was demonstrated that the strong interaction between the
32 biomolecules in solution modified the membrane fouling and had a clear impact on the

33 membrane permeation and selectivity, similarly to the real mixtures. The dynamic filtration was
34 tested to enhance the performances compared to cross-flow filtration. The higher shear rate
35 favored an increase in flux and therefore productivity, but did not modify membrane selectivity.
36 It was therefore demonstrated that the interaction between molecules existing in the cell or
37 generated during the cell disruption drives the separation efficiency and should be mitigated in
38 future works to allow the whole biomass biorefining.

39 **Keywords**

40 Cross-flow filtration, shear-enhanced filtration, microalgae biorefinery, lipids proteins
41 interaction, Oil-in-water emulsion

42 **1. Introduction**

43 Nowadays, microalgae cultivation and valorization have become an attracting research
44 topic as its potential has been successfully demonstrated in various sectors, such as energy,
45 cosmetics, pharmaceuticals, food and feed. Intensive research has been performed for the
46 optimization of microalgae production [1–5]. Several microalgae strains, *Chlorella vulgaris* for
47 example, are rich in proteins and are considered as a new protein resource for food and feed.
48 Other strains, like *Parachlorella kessleri*, were identified as able to enhance the lipid storage
49 under specific culture conditions, these lipids has a great potential for biofuel conversion after
50 extraction [6–8].

51 The microalgae biorefinery in wet processing was proposed by several authors [9–12]
52 in order to avoid the conventional biomass drying step before extraction, which is either high
53 energy consuming or physical-biological properties affecting. The eco-friendly wet processing
54 involves four major operations: the harvesting step to concentrate the biomass and to recycle
55 the culture medium, the biomass cell disruption to release the intracellular compounds in the
56 aqueous phase, the clarification of the microalgae extract to remove the solid cell fragments,
57 and the final separation to fractionate and purify the hydrophobic (lipids and parts of pigments)
58 and hydrophilic compounds (ash, soluble proteins and carbohydrates).

59 Considering cell disruption, the bead milling has been deeply studied due to its high
60 efficiency at high biomass concentrations (30-100 g·L⁻¹) [13,14]. For the separation of
61 biomolecules dispersed in the remaining aqueous phase after bead milling, membrane
62 processing is a suitable technology due to the simplicity in operation (without solvent use), the
63 flexibility for implementation (wide range of materials, cut-off and geometries) and the
64 potential for large volume treatment [15,16]. Several studies on microalgae biorefinery

65 downstream processes have been focused on the membrane separation or purification process
66 [14,17–19]. The ultrafiltration membranes with a molar weight cut off up to 300 kDa were
67 mostly used to concentrate the biomolecules from the aqueous extract. But when the objective
68 is to let the hydrophilic compounds permeate through the membrane, the performances are
69 much lower, most of the lipids are fully retained up to 100 %, but the protein recovery yields
70 in the permeate is very low (retention rate above 80 %), even with microfiltration membranes.
71 This can be related to the difficulty of coupling bead milling (BM) and membrane filtration.
72 Indeed, during BM process for cell disruption, the applied mechanical force has no specific
73 destruction target. As a consequence, the release of amphiphilic molecules (proteins, polar
74 lipids, etc.) and neutral lipid droplets (triacylglycerides, free fatty acids) in the presence of high
75 shear rate brings together the conditions to produce a stable emulsion [20–23]. This new stable
76 system composed of complex colloidal structures will become a major obstacle in the
77 downstream separation and purification.

78 Thus, the key point to optimize the coupling of BM and membrane processes is to
79 understand the nature of the released compounds, the interactions between the compounds and
80 compounds/membrane under processing conditions, and how these interactions impact the
81 filtration performances (selectivity and permeation flux).

82 The deepening of the impact of the composition of the complex aqueous microalgae
83 extracts on the microfiltration performances necessitates large volumes of extracts
84 corresponding to several m³ of microalgae cultures. The production of such large quantities of
85 microalgae aqueous extracts would necessitate both high investment and skilled labor,
86 especially in the case of strains cultivated in starving conditions to orientate their metabolism
87 (like *Parachlorella kessleri* to produce lipids). The current cultivation processes do not allow
88 to fulfill these criteria. Moreover, the different batches of biomass and their storage would
89 induce variability in the filtered solution as deduced from already available characterizations
90 (composition, organization, etc.) [13,22]. Thus, in this work, the strategy was to elaborate
91 controllable model mixtures with an increasing complexity to deepen the study of the
92 interaction and their impact on the process.

93 The objective was to study the impact of the lipid-protein interactions on the
94 performances of the microalgae extracts microfiltration, using accessible methods to go towards
95 local understanding. Three model mixtures were formulated with composition close to real
96 microalgae extracts, containing only proteins (MM_P), only lipids (MM_L) or both (MM_{P/L}), and
97 characterized by electrophoresis and particle size distribution. Then they were filtrated with a

98 polyethersulfone (PES) membrane with a mean pore diameter of 0.1 μm, the performances were
99 compared to the filtration of real microalgae extracts, and the fouling characterized using
100 fouling resistance measurement and ATR-FTIR. The impact of the interaction between proteins
101 and lipids on membrane filtration performances was highlighted (retention rate, flux, fouling
102 characterization). The effect of the filtration hydrodynamics on the membrane permeability and
103 the selectivity with such complex mixtures was also investigated.

104 **2. Materials and Methods**

105 **2.1. Preparation of the model mixtures**

106 The model mixture preparation included three steps: the aqueous phase preparation, the
107 oily phase preparation and the emulsification process.

108 *2.1.1. Aqueous phase preparation*

109 For MM_L, the aqueous phase contained only a diluted phosphate buffer (pH 7.4,
110 Conductivity 790 μS·cm⁻¹, close to *Parachlorella kessleri* culture medium properties). For
111 MM_P and MM_{P/L}, the selected vegetable protein product was firstly dispersed at 12.5 g·L⁻¹ in
112 phosphate buffer (pH 7.4, Conductivity 790 μS·cm⁻¹) and continuously stirred at 700 rpm for
113 24 hours at room temperature to reach maximum solubilization. Then the soluble protein
114 fraction was recovered by centrifugation at 12 000 g for 15 min and the soluble protein
115 concentration was adjusted at 0.7 g·L⁻¹ by the addition of a phosphate buffer.

116 *2.1.2. Oily phase preparation*

117 A concentrated lipid phase was prepared to ensure the appropriate polar and neutral
118 lipids' balance as follows: 2.6 g lecithin extract and 17.4 g vegetable oils were homogenized
119 using an ultrasonic processor (VCX130 PB Vibra-Cell™, Sonics, USA) at 65 % amplitude for
120 6 min.

121 *2.1.3. Emulsification process*

122 First of all, the appropriate volume of the lipid phase was added to the aqueous in order
123 to achieve a protein/lipid concentration ratio of 1/2, and this solution was pre-emulsified by a
124 rotor-stator T25 homogenisator (T25 Digital ULTRA-TURRAX®, IKA®, Germany) at 10 000
125 rpm during 2 min to avoid the oily phase float on the surface of the aqueous phase during the
126 emulsification. Then it was emulsified with a continuous rotor-stator T18 (T18 Digital ULTRA-

127 TURRAX®, IKA®, Germany) at 19 000 rpm for 45 min. The stability of the model mixtures
128 after preparation was followed by granulometry (Mastersizer 3 000, Malvern Panalytical, UK).

129 **2.2. Real and model mixtures characterization technics**

130 The dry matter was determined by drying the samples up to constant mass in an oven at
131 105 ± 5 °C. The soluble proteins were quantified by a BCA Protein Assay kit (Thermo
132 Scientific) (the proteins suspended in the supernatant at 12 000 g were named “soluble” even if
133 colloids can be present). The total lipids were determined by a gas chromatography-flame
134 ionization detector and polar lipid classes were quantified by a high-performance thin-layer
135 chromatography [22].

136 The emulsion droplet size distributions were analyzed by a MASTERSIZER 3000 size
137 analyzer (Malvern Panalytical, UK), the particles refractive index was set at 1.47 for MM_P and
138 MM_{L/P}, at 1.465 for MM_P and at 1.430 for the real mixtures from *Parachlorella vulgaris*. The
139 particulate absorption index was set at 0.01[24–27].

140 Two types of electrophoresis, Native-PAGE (Native PolyAcrylamide Gel
141 Electrophoresis) and SDS-PAGE (Sodium DodecylSulphate PolyAcrylamide Gel
142 Electrophoresis) were carried out to determine the molecular weight of the native proteins and
143 their subunits. The Native-PAGE was performed with a 12 % native polyacrylamide handmade
144 gel with 10-1 200 kDa NativeMark Unstained protein standard (ThermoFisher). For SDS-
145 PAGE, the proteins were denatured in a SDS sample buffer at 95 °C during 3 min; for
146 electrophoresis, a Bolt™ 10 % Bris-tris Plus Gels and 40-300 kDa Spectra™ Multicolor High
147 Range Protein Ladder standard (ThermoFisher) were used.

148 All the values resulting from the samples analysis (dry matter, lipids and proteins
149 analysis) were calculated by the means of triplicate measurements. The standard errors (SE)
150 presented in the results were calculated as the standard deviations of the triplicate divided by
151 $\sqrt{3}$. The combined standard error of any y value was calculated by Equation 1 for additions or
152 Equation 2 for multiplications or divisions of two variables X₁ and X₂:

153 If $y = X_1 + X_2$,

154
$$SE_y = \sqrt{SE_{X_1}^2 + SE_{X_2}^2} \quad \text{Equation 1}$$

155 If $y = X_1^{\pm 1} \times X_2^{\pm 1}$,

156
$$SE_y = y \cdot \sqrt{\left[\frac{SE_{X1}}{X1}\right]^2 + \left[\frac{SE_{X2}}{X2}\right]^2}$$
 Equation 2

157 **2.3. Membrane filtration**

158 *2.3.1. Cross-flow filtration pilot*

159 The membrane with an effective filtering area of 130 cm² was mounted in a Rayflow
 160 X100 module (Rhodia, Orelis) equipped with a 1.5 mm sealing gasket corresponding to the
 161 liquid channel thickness. The cross-flow filtration was performed thanks to a peristaltic pump
 162 (Masterflex I/P77600-62, Cole Parmer, USA). A valve on the retentate outlet pipe allows
 163 mastering the transmembrane pressure (TMP), calculated as the average of the pressures at the
 164 inlet and outlet measured by 2 pressure sensors (± 0.01 bar). The feed solution temperature was
 165 maintained with a regulated warming plate. In order to enhance the back-transport mechanisms
 166 near the membrane surface, a spacer of 1.5 mm (reference 46 mil) was used and the apparent
 167 cross-flow velocity was estimated at $v_{app}=0.8$ m.s⁻¹. The permeate was continuously weighted
 168 to measure the permeate flow (+/- 1-3% in DF and 5-8% in CF due to the peristaltic pump).
 169 All the data were collected with a Labview software.

170 *2.3.2. Rotating disc filtration pilot*

171 The dynamic filtration experiments were carried out using a rotating disk dynamic
 172 filtration system. As described by Frappart et al. 2011 and Bouzerar, Ding, et Jaffrin 2000
 173 [29,30], the membrane was installed into the cylindrical chamber in front of the disk, and the
 174 effective membrane area was 188 cm². The feed (stirred and temperature controlled) was sent
 175 to the filtration module by a peristaltic pump (Masterflex I/P77600-62, Cole Parmer, USA).
 176 The peripheral pressure (Pc) was adjusted by a valve on the retentate outlet and measured at the
 177 top of the cylindrical housing by a pressure sensor. Rotational disk speed could be adjusted
 178 from 0 to 3 000 rpm, and the disk was equipped with radial vanes. The shear rate was calculated
 179 as a function of rotation velocity and disk profile as described by Bouzerar, Ding, et Jaffrin
 180 2000 [30] (Equation 3).

181 In order to study the impact of hydrodynamic conditions on the filtration performances,
 182 two hydrodynamic conditions were tested in DF: 16 000 s⁻¹ (γ_1) and 66 000 s⁻¹ (γ_2).

183
$$\gamma = 0.0296r^{8/5}(k\omega)^{9/5}v^{-4/5}$$
 Equation 3

184 Where: ω is the disk angular velocity (rad s^{-1}), k is the velocity factor which was 0.89
185 in our case for a disk equipped with 6 mm vanes, ν the kinematic viscosity ($\text{m}^2\cdot\text{s}^{-1}$) and r the
186 distance from the center, where the maximum shear rate value for tested two hydrodynamic
187 conditions γ_1 and γ_2 were calculated with the disk radius equal to 0.0725 m, at the membrane
188 periphery.

189 2.3.3. *Membrane preparation prior filtration*

190 For each experiment, a new organic microfiltration membrane PES 0.1 μm (K618,
191 KOCH Membrane Systems, USA) was previously conditioned as follows: it was rinsed with
192 deionized (DI) water to remove the preservative, cleaned with 100 ppm chlorine solution under
193 alkaline conditions (pH 10) for 30 min at room temperature (23 °C), then cleaned with 0.2 %_{v/v}
194 Ultrasil 110 (Ecolab, USA) for 30 min at 45 °C, and finally rinsed with DI water during 35 min.
195 after the cleaning and before filtration, the membrane was compacted at 30 °C under a
196 transmembrane pressure TMP of 1 bar until the permeate flow was stabilized. The membrane
197 initial water permeability was measured for each coupon, before each filtration.

198 Due to the heterogeneity of the membrane material different values of the water
199 permeability were observed on the different membrane 130 cm^2 coupons of a same lot of PES
200 membranes, which was in accordance with the membrane pore size distribution obtained by
201 scanning electron microscopy (the majority of the pores had a diameter between 50 and 110 nm
202 for the membranes with a higher water flux, and between 20 and 50 nm for the others).
203 Therefore, during the flux results analysis, the permeate flux was always normalized to the
204 initial DI water flux of the membrane before filtration.

205 2.3.4. *Filtration of MM and RM*

206 2.3.4.1. Determination of critical conditions with MM

207 First of all, in order to limit the membrane overall fouling during the set of experiences
208 in concentration mode (increasing volume reduction ratio VRR), the critical pressure of the
209 most complex model mixture $\text{MM}_{\text{P/L}}$ was determined both in CF and in γ_1 hydrodynamic
210 conditions for DF. The critical pressure, corresponding to the lowest TMP to avoid high
211 irreversible fouling in given hydrodynamic conditions, was determined in batch mode with full
212 recycling of both retentate and permeate to the feed tank (30 °C). The dry matter concentration
213 was thus maintained constant and VRR=1 was remained. Accordingly, TMP was voluntary
214 gradually increased from 0.1 to 0.5 bar for CF and from 0.1 to 1 bar for DF (0.1-0.2 bar each

215 step). The stabilized permeate flux was measured for each TMP. The critical conditions (critical
216 TMP, critical flux) were deduced from the J_p vs TMP plot and correspond to the last point
217 belonging to the first linear part of the plot. The critical pressure was 0.50 bar for CF and 0.54
218 for DF, respectively.

219 2.3.4.2. Filtration in concentration mode below critical TMP

220 The concentration process was carried out with 3L of the solution at 30 °C regardless
221 of CF or DF filtration mode, the VRR was increased up to 3 as follows: the retentate was fully
222 recycled into the feed tank while the permeate was continuously extracted. The working TMP
223 was chosen at 90 % lower than the critical pressure and set at 0.45 bar for CF and 0.50 bar for
224 DF. For each filtration samples of the feed, retentate and instantaneous permeate were regularly
225 collected and subsequently analyzed. Two hydrodynamic conditions were tested for DF (γ_1 at
226 $16\,000\text{ s}^{-1}$ and γ_2 $66\,000\text{ s}^{-1}$): γ_1 was used for each model mixture, and only $MM_{P/L}$ was also
227 filtrated with γ_2 to understand the impact of the shear stress increasing. Each experiment with
228 model mixtures was repeated at least twice. For RM, a subcritical TMP of 0.30 bar was selected
229 for both CF and DF according to previous determination and following a similar protocol. RM
230 was filtrated once in CF and once in DF under γ_1 hydrodynamic condition as well to compare
231 with the results of MM.

232 2.3.5. Membrane rinsing and cleaning after MM and RM filtration.

233 After filtration, a simple water rinsing with 5 L at 30°C in an open circuit was carried
234 out in order to remove the physically reversible fouling, then the membrane water permeability
235 was measured to determine the fouling resistance R_f including both the chemically reversible
236 (removed by chemical cleaning) and the irreversible (remaining after cleaning) fouling
237 (Equation 4).

$$238 \quad R_f = R_t - R_m = \frac{TMP}{\mu} \left(\frac{1}{J_{wf}} - \frac{1}{J_{wi}} \right) \quad \text{Equation 4}$$

239 Where: R_t represents the total resistance calculated from water permeability
240 measurement (water flux J_{wf}) after the concentration experiment and the water rinsing, and R_m
241 the membrane resistance calculated by water permeability measured on the pristine membrane
242 (water flux J_{wi}).

243 After rinsing, the membrane was removed from the pilot and prepared for the
244 subsequent membrane fouling characterization.

2.4. ATR-FTIR membrane fouling characterization

The membrane lipids fouling accumulation was characterized by ATR-FTIR. Membrane samples were carefully dried under dynamic vacuum at least three days before ATR-FTIR registration, in order to remove adsorbed water and avoid the corresponding absorption band (large band around 3300 cm⁻¹ and harmonic band at 1660 cm⁻¹).

For the sake of accuracy, several spectra were registered for each membrane. For cross-flow, the mean value of 4 spectra measured in the center of the membrane is depicted in the results. For dynamic filtration, the values from the center of the membrane, the intermediate annular and the external annular bands are shown each one corresponding to the average of 4 spectra (see Figure 5).

The ATR-FTIR spectra were registered with a Spectrum 100 (Perkin Elmer) spectrometer using the Spectrum for Windows software (5.3) either for data acquisition or spectra treatment. The spectrometer was equipped with an ATR accessory having a diamond monoreflexion crystal (1.8 mm size) with an incidence angle of 45°. The background was recorded in the air and each spectrum was the accumulation of 20 scans with 2 cm⁻¹ resolution in the 4000-600 cm⁻¹ range. The band height H^x at the x wavenumber were then measured on the raw spectra after setting the baseline between 2240 and 2060 cm⁻¹ (region without any absorption bands of the membrane) adapting the procedure reported in [31,32].

In absence of calibration which is too long to establish, semi-quantification was carried out inspired by the methodology proposed by *Delaunay et al. 2008* for protein quantification [33] and as reported in [31,32]. The relative concentration of lipids deposited on the membrane was evaluated through calculation of $\frac{H_{lipids}}{H_{PES}}$ ratios, H_{lipids} being a band of high intensity typical of the lipids of the oil-in-water emulsion and H_{PES} being a band of high intensity typical of PES used as internal standard. H_{lipids} was set at 1744 cm⁻¹ (C=O stretching, fatty acid triglycerides or phospholipids esters), and H_{PES} was selected at 1576 cm⁻¹ (C=C) for PES membrane both being of high intensity and without any overlapping.

271 **3. Results and Discussion**

272 **3.1. Formulation of the model mixtures**

273 **3.1.1. Selection of the model mixtures ingredients**

274 *3.1.1.1. Neutral and polar lipids in model mixtures*

275 In the supernatant obtained from nitrogen starved *Parachlorella kessleri* after cell
276 disruption and centrifugation, the total lipids of the supernatant contain around 46%
277 triacylglycerides (TAG), 44% other neutral lipids (mainly free fatty acids FFA), and 10% of
278 polar lipids (6% glycolipids and 4% phospholipids) playing the role of an emulsifier [22]. In
279 model mixtures, a blend of vegetable oils was formulated to represent the neutral lipids (mostly
280 TAG) present in the discontinuous oily phase of a real mixture [34].

281 Polar lipids, especially phospholipids are natural amphiphilic molecules and are the
282 major compounds of the cell membrane. Phospholipids are commonly used as emulsifiers
283 because they have hydrophobic fatty acid tail groups and hydrophilic head groups containing
284 phosphoric acid esterified with glycerol and other substitutes [35]. Under oil-in-water (O/W)
285 emulsion, the high flexibility of phospholipids due to their small size and clearly defined two
286 opposite affinity parts, promote a rapid interface arrangement compared to proteins [21].

287 To represent the polar lipids (phospholipids and glycolipids), a lecithin extract from
288 soybeans (VWR CHEMICALS, USA) which contains 97.6%_{DM} of polar lipids and 2.4%_{DM} of
289 proteins was selected based on the polar lipids compositions and purity of this product.

290 The comparison of the polar lipids characteristics between the real mixture – the
291 supernatant – and lecithin extract is presented in Table 1. The supernatant contains two main
292 families of polar lipids with surfactant properties: glycolipids and phospholipids. For the
293 phospholipids fraction, the supernatant contained mainly 28 % PC and 13 % PI, compared to
294 the 31 % PC and 38 % PI for the lecithin extract, while for glycolipids, 7 % MGDG and 9 %
295 DGDG for the supernatant and 14 % and 1 % for the lecithin extract.

296 Despite those inevitable differences between the two bioresources, to ensure a stable
297 emulsion system, the lecithin extract was chosen as an appropriate model of lipid-based
298 emulsifier for model mixtures formulation. The ratio between neutral lipids (TAG from
299 vegetable oils blend) and polar lipids (Lecithin) in model mixture was fixed as 87 % neutral
300 lipids and 13 % polar lipids, similar to the proportion in the supernatant (90 % neutral lipids
301 and 10 % polar lipids).

Table 1. Characteristics of polar lipids from supernatant and lecithin extract*

Full name	Abbreviation	Supernatant	Lecithin extract	
		g·g ⁻¹ polar lipids	g·g ⁻¹ polar lipids	% DM
Lysophosphatidylcholine	LPC	11	8.0 ± 2.4	7.8 ± 2.4
Phosphatidylcholine	PC	28	31.6 ± 0.9	30.8 ± 0.9
Phosphatidylinositol	PI	13	38.7 ± 1.0	37.8 ± 1.0
Cardiolipid	CL	6	0.7 ± 0.1	0.7 ± 0.1
Phosphatidylethanolamine	PE	1	DT	DT
Phosphatidylglycerol	PG	2	DT	DT
Sulfoquinovosyldiacylglycerol	SQDG	23	DT	DT
Sphingomyeline	SM	DT	0.7 ± 0.4	0.7 ± 0.3
Monogalactosyldiacylglycerol	MGDG	7	13.8 ± 4.2	13.5 ± 4.1
Digalactosidediacylglycerol	DGDG	9	1.3 ± 0.1	1.3 ± 0.1

303 * DT: detected, no standard for quantification.

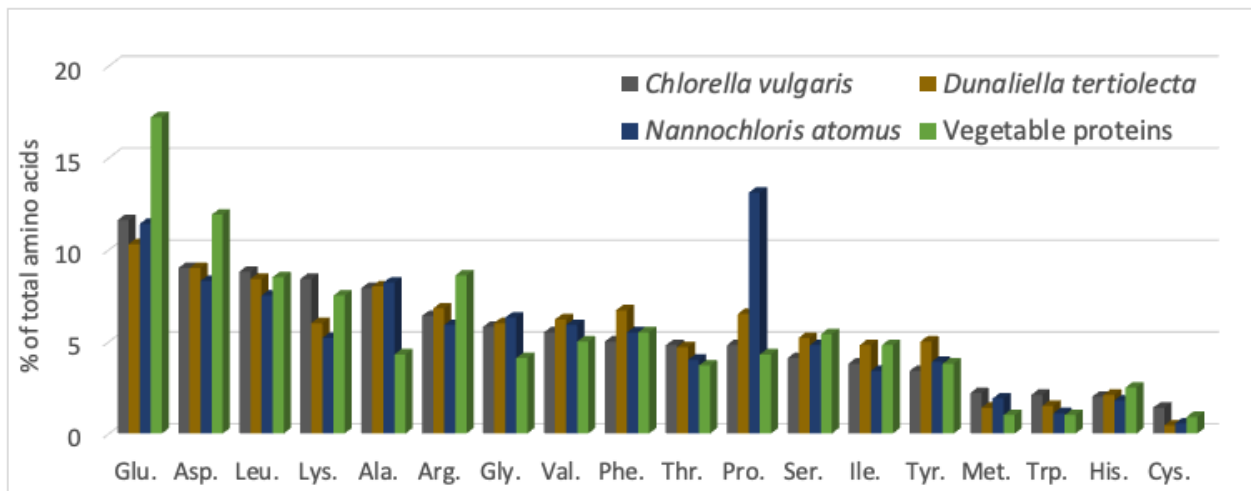
304 3.1.1.2. Proteins in model mixtures

305 The proteins, which are macromolecules with various functions in the microalgae cells,
 306 are more difficult to characterize. The hydrophobic or hydrophilic groups in proteins are
 307 determined by the primary sequence of the amino acids, some of them being exposed or hidden
 308 with respect to the tertiary structure folding and even their quaternary structure corresponding
 309 to aggregation in soluble state. However, this native conformation can be modified by hydrogen
 310 bonds, hydrophobic interactions and ion-ion interactions with substances of their physico-
 311 chemical environment [36]. Microalgae proteins contain an important ratio of amino acids with
 312 hydrophobic side groups as isoleucine, phenylalanine and leucine [37]. In an O/W emulsion,
 313 amphiphilic proteins may adsorb onto an oil-water interface, reorganize their hydrophobic
 314 groups into oil droplets and hydrophilic groups into the aqueous phase [21]. In consequence,
 315 the protein molecules may partially be denatured or unfolded during the reorganization
 316 [21,38,39]. Accordingly, the variation of the conformation is protein dependent and would
 317 strongly depend on the fraction of hydrophobic amino acids (number and spatial repartition in
 318 the primary sequence) and on the surface of interactions with the outer environment that result
 319 from complex equilibria.

320 Since the amino acid profile of proteins from *Parachlorella kessleri* was not described
 321 in literature, the protein extracts from microalgae *Chlorella vulgaris*, *Dunaliella tertiolecta* and
 322 *Nannochloris atomus*, with the same chlorophyte phylum as *Parachlorella kessleri*, were
 323 compared with a selection of vegetable proteins available in appropriate quantity on the market.

324 An organic vegetable protein product was then selected. The amino acid profiles are compared
 325 in Figure 1. Microalgae contain an important ratio of leucine (Leu), phenylalanine (Phe),
 326 isoleucine (Ile), amino acids with amphiphilic properties [21,37–39]. The selected vegetable
 327 protein product contains these amino acids in similar proportions and was selected accordingly.
 328 This selected vegetable protein source contains 85 % proteins, 9.4 % lipids, 0.9 % fibers and
 329 less than 0.5 % carbohydrates. The soluble proteins were extracted to represent the soluble
 330 proteins from a real mixture (supernatant).

331



332

333

Figure 1. Amino acid profile comparison between microalgae and vegetable proteins.(Becker, 2007)

334

3.1.2. Model mixture formulation strategy

335

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338

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341

The composition of the real mixture (RM) and the model mixture (MM) are described in Table 2. The RM were the supernatant fraction recovered after the bead milling and centrifugation 3000 g of a *Parachlorella kessleri* biomass at 3.7 g·L⁻¹ of dry matter. RM contains 0.31 g·L⁻¹ of lipids, 0.20 g·L⁻¹ of proteins and 0.72 g·L⁻¹ of carbohydrates. The proportions of proteins, lipids and carbohydrates in RM are from the expected ones [40] and could be related to difficulties in the culture of *Parachlorella kessleri* in starving conditions at a large scale.

342

343

344

345

346

347

It was demonstrated that a bead milling at a higher biomass concentration is more energetic efficient (up to 100 g·L⁻¹)[13]. However, in this work, the experiments of RM with higher biomass concentration could not be realized due to the limitation of biomass feedstock. Therefore, the MM was specifically set to be more concentrated than RM but the same lipids-proteins ratio was preserved in MM as in RM. The concentrations of lipids and soluble proteins were 5 times higher than in RM not only to mimic microalgae extracts obtained after the bead

348 milling at a higher concentration, but also to simulate the performance of the membrane under
 349 harsher conditions. As shown in Table 2, MM_L contains around 0.15 %_{w/v} mixture of neutral
 350 and polar lipids, MM_P contains 0.075 %_{w/v} soluble vegetable proteins and MM_{P/L} contains 0.15
 351 %_{w/v} of total lipids and 0.075 %_{w/v} soluble vegetable proteins.

352 The RM also contain carbohydrates but their nature and quantity strongly varies
 353 depending on the culture conditions. It was chosen as a first approach not to introduce such a
 354 fraction in the MM because these biomolecules are not currently well described. The
 355 development of a corresponding MM would necessitate a deep characterization of the complex
 356 carbohydrates contained in the considered microalgae, which have been limited by time in this
 357 study. However the comparison between RM and MM have brought indirect understanding on
 358 their potential role.

359 *Table 2. Comparison of formulated model mixtures (MM) compositions with real mixtures (RM)**

	Measurement			
	DM g·L ⁻¹	Lipids g·L ⁻¹	Soluble proteins g·L ⁻¹	Carbohy drates g·L ⁻¹
MM _L	2.5±0.2	1.43±0.02	-	0
MM _P	2.0±0.2	-	0.70±0.10	0.07
MM _{P/L}	3.6±0.2	1.41±0.02	0.70±0.10	0.07
RM	2.5±0.1	0.31±0.01	0.20±0.01	0.72

360 *RM: Real mixtures were the supernatants from bead milling and centrifugation of the biomass *Parachlorella*
 361 *kessleri* 3.7 g·L⁻¹ (ash-free dry matter). DM presented in the table are total dry matter including ash. (-) measured,
 362 not detected.

363

364 **3.1.3. Comparison of the model mixtures and microalgae extracts**

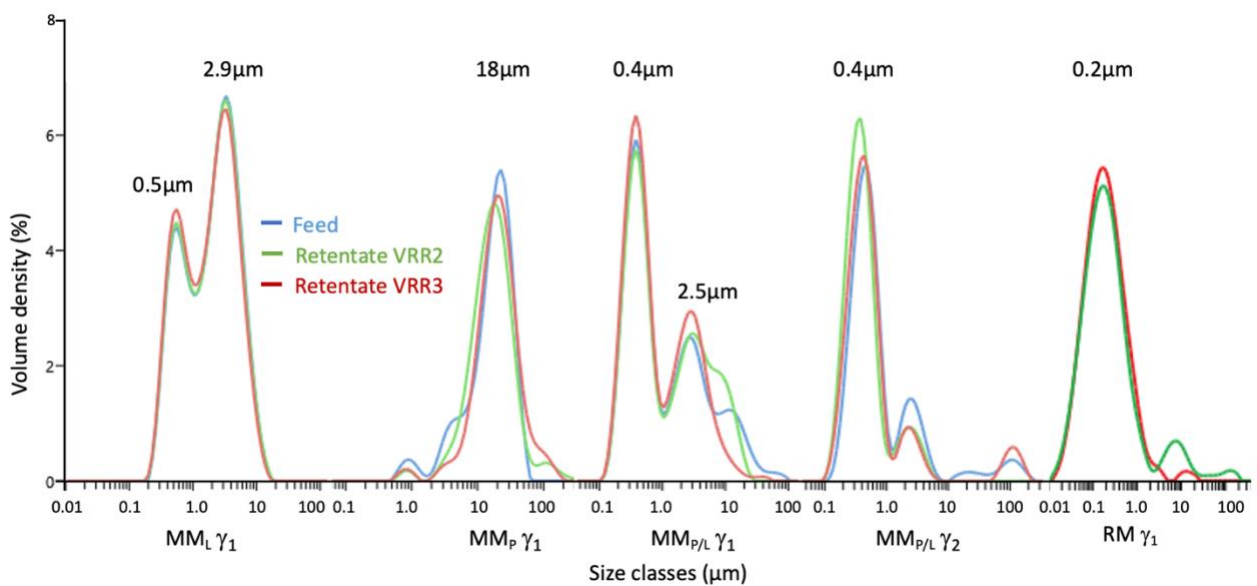
365 After model mixtures formulation, the composition and the organization of the
 366 compounds in the model and real mixtures were compared using different characterization
 367 technics as described in 2.3.

368 *3.1.3.1. Particle size distribution*

369 Figure 2 compares the particle volume size distributions between the real mixture and
 370 the model mixtures before and during filtration, measured by granulometry on Mastersizer
 371 3000. Regarding the real mixture RM, the particle size mainly ranged from 0.01 to 2.0 µm,
 372 some large objects, around 10-100 µm, were also detected, which may be the cell fragments
 373 recovered in the supernatant or aggregates after centrifugation. Regarding the particle size of
 374 MM_L containing only polar and neutral lipids, the particles were distributed between 0.1-10

375 μm , with two main modes at $0.5 \mu\text{m}$ and $2.9 \mu\text{m}$, corresponding for the smallest to micelles or
 376 vesicles of polar lipids, and for the largest to O/W droplets. For MM_P , the main mode was at 18
 377 μm , which means a part of the proteins were aggregated into large particles and were not
 378 eliminated by the former centrifugation. The free proteins can not be measured with the
 379 granulometry method. The aggregation was also observed by Linder, 2009 and Stradner et al.,
 380 2004 [41,42]: they observed that the proteins form monolayers like supramolecular clusters and
 381 micelles in aqueous bulk phases. Surprisingly, in $\text{MM}_{P/L}$, with mixture of soluble proteins, polar
 382 lipids and neutral lipids, the particle volume size distributions showed mainly two modes at 0.4
 383 μm and $2.5 \mu\text{m}$, smaller than the aggregates in MM_P . The protein-lipid mixture is milky white
 384 and opaque, but clear and homogeneous.

385 According to Courthaudon et al., 1991; Damodaran, 2005 and Euston et al., 1995
 386 [21,43,44], when polar lipids, like lecithin, and proteins are mixed together as emulsifiers before
 387 emulsification process, those molecules compete with each other for adsorption on the O/W
 388 interface and form multi-emulsifier micelles. This competitive adsorption varies with the type
 389 and ratio of the two emulsifiers. Their interaction (competition or coexistence) will impact the
 390 characteristics of the emulsion droplets. According to the particle size distributions of the
 391 model mixtures, it appears here that the large protein aggregates were dissociated. They could
 392 be either on the lipid droplets, in micelles or partially dissolved. However the particle size
 393 distribution of the $\text{MM}_{P/L}$ is the nearest of the RM one. Thus a clear interaction between proteins
 394 and lipids was highlighted in $\text{MM}_{P/L}$.



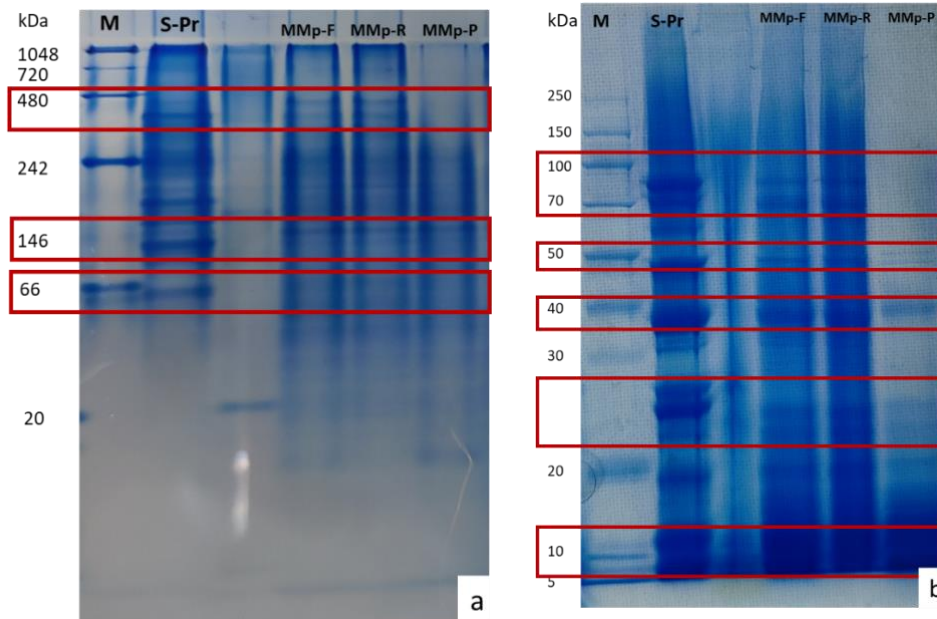
395

396 *Figure 2. Particle size volume distribution comparison between model mixtures and real mixtures before and*
397 *during rotating disc filtration. MM: Model Mixture, RM: Real mixture, γ : filtration hydrodynamic conditions γ_1*
398 *at $16\,000\ s^{-1}$ and $\gamma_2\ 66\,000\ s^{-1}$*

399 3.1.3.2. Electrophoresis analysis

400 In this paragraph, the objective is to verify if the vegetable protein is an acceptable
401 model for microalgae proteins. Figure 3 shows the results of electrophoresis in Native-PAGE
402 and SDS-PAGE in order to compare the proteins and their subunit sizes between extracted
403 vegetable proteins and microalgae proteins. According to Alavijeh et al., 2020; Günerken et al.,
404 2016; Teuling et al., 2017 [45–47], microalgae contain an important quantity of RubisCO, a
405 photosynthetic enzyme for carbon fixation, with a native protein size between 550 kDa-480
406 kDa depending on the strain [47]. It was confirmed by Liu et al. that vegetable proteins also
407 contain two protein bands close to 480 kDa (Figure 3.a).

408 In Figure 3b shows the results of SDS-PAGE for vegetable protein subunits
409 characterization. Compared with the microalgae SDS-PAGE from Teuling et al., 2017, we
410 observed that extracted vegetable protein bands were similar to those of microalgae *Arthrospira*
411 *sp.* and *Nannochloropsis sp.*, where two bands 55-65 kDa and 10-20 kDa were identified, which
412 could correspond to 8 large subunits and 8 small subunits of Rubisco. Two bands corresponding
413 to units of 40 kDa and 20-30 kDa were also present in microalgae and vegetable proteins. Those
414 results confirmed that vegetable proteins were an acceptable model to represent the microalgae
415 proteins. We can suppose that the interactions between lipids and proteins in the model and real
416 mixtures could be similar.



417 *Figure 3. Protein electrophoresis results of extracted soluble vegetable proteins. a: Native-PAGE result from*
 418 *vegetable protein; b: SDS-PAGE results between vegetable protein. (M: marker, S-Pr: extracted soluble*
 419 *vegetable protein, MM_p-F/R/P: feed, retentate or permeate from filtration of MM_p sampled at VRR3)*

420 **3.2.Filtration performances studies of the model mixtures and real** 421 **extracts**

422 In the following paragraphs, the filtration performances (permeation flux and
 423 selectivity) are compared for the filtration of the three model and the real microalgae extracts.
 424 The analysis complexity is highlighted due to the contrary role of biomolecules on membrane
 425 hydrophilisation and fouling. The impact of the lipid-protein interaction on the membrane
 426 fouling is demonstrated, its mitigation by shear rate evaluated and the membrane selectivity in
 427 the different conditions compared.

428 *3.2.1. Competition between membrane fouling and hydrophilization by the* 429 *biomolecules*

430 The hydraulic performances of the membrane before, during and after the filtration of
 431 model and real mixtures at 30 °C are shown in Table 4 and 5. Due to heterogeneity of membrane
 432 porous material, the membrane water flux before concentration experiment (J_{wi}) ranged from
 433 22 to 61 L·h⁻¹·m⁻² for CF and 19 to 66 L·h⁻¹·m⁻² for DF.

434 In CF, considering the membranes with the higher initial water flux (above $30 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$)
435 ²), the filtration of the model mixtures led to a decrease of the permeate flux and the water
436 permeability after rinsing was lower than the initial one. A large quantity of biomolecules
437 probably accumulated on the membrane and blocked pores. For membranes with a lower initial
438 water flux (below $30 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$), the enhancement of the hydrophilicity had a preponderant effect
439 on the permeation, leading to a normalized membrane permeability $J_{\text{MMx}}/J_{\text{Wi}} > 1$. Thus the
440 evolution of the flux of a given membrane results from a compromise between the membrane
441 hydrophilicity and fouling. In CF, the highest permeation flux was obtained with MM_P .

442 In DF, regarding the values of the normalized membrane permeability during the
443 concentration process of model mixture $J_{\text{MMx}}/J_{\text{Wi}}$ VRR1-3, the flux was always superior to the
444 water flux ($J_{\text{MMx}}/J_{\text{Wi}} > 1$). This is coherent with the results from Villafaña-López et al., 2019
445 [48], even if the authors have worked with a higher shear rate. On the membrane surface, the
446 accumulation of a part of the biomolecules increase the hydrophilicity of the porous material.
447 The partially hydrophobic properties of virgin PES membranes were already described in
448 literature [48–50] and it was already shown on the same membrane K618 (Koch) that polar
449 compounds like proteins could enhance or reduce water permeation depending on the fouling
450 structure [51].

451 After water rinsing, the final water flux J_{Wf} was lower than the initial water flux and the
452 filtration flux. It means that the physically reversible fouling made the membrane more
453 hydrophilic, but once it was removed, a residual fouling induced a lower permeability. The
454 impact of this fouling was hidden by the rise of hydrophilicity. Regarding the experiments
455 performed in the same hydrodynamic conditions γ_1 with membrane presenting similar initial
456 water flux (above $30 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$), we observed that the model mixture MML , with only 0.15 % of
457 lipids and without proteins, has the highest normalized flux compared to the model mixtures
458 containing proteins. MM_P has the lowest. This implies that the hydrophilization of the
459 membrane by MML was improved, probably because of the polar lipids, which adsorbed to the
460 PES polymer, and/or that the fouling by lipids is limited. The polar lipids are smaller and more
461 mobiles than proteins, thus, it is probably easier for them to attach to the membrane surface and
462 to modify the membrane hydrophilicity without blocking the pores.

463 Regarding the RM, which is 5 times less concentrated (protein and lipids) than the MM,
464 for CF, the permeation flux, the flux ratio $J_{\text{RM}}/J_{\text{Wi}}$ and the J_{Wf} are in the same range as the values
465 obtained with the $\text{MM}_{P/L}$ despite the protein and lipid concentration differences. For DF, $J_{\text{RM}}/$

466 J_{wi} was still >1 but lower than with the MM. A lower availability of the amphiphilic molecules
 467 involved in more complicated aggregates could explain this difference or the rise of membrane
 468 fouling due to the presence of other biomolecules like carbohydrates could be the reason.

469

470 *Table 4. Hydraulic performance of the membrane before, during and after CF at 30 °C and 0.45 bar **

N° Assay for CF	J_{wi} $L \cdot h^{-1} \cdot m^{-2}$	J_{wf} $L \cdot h^{-1} \cdot m^{-2}$	J_{MMx} VRR1-3 $L \cdot h^{-1} \cdot m^{-2}$	J_{MMx} / J_{wi} VRR1-3
MM _L	40		39-36	0.9-1.0
MM _L '	61	48	50-51	0.7-0.8
MM _P	44	34	44-43	0.9-1.0
MM _P '	27	32	32-38	1.2-1.4
MM _{P/L}	56	58	49-47	0.8-0.9
MM _{P/L} '	56	38	44-36	0.6-0.8
MM _{P/L} ''	22	22	26-25	1.1-1.2
RM	38	44	35-25	0.7-0.9

471 * J_W Membrane water flux before (i) or after (f) concentration experience. J_{MMx} VRR1-3: membrane model mixture
 472 flux variation during concentration, values calculated from VRR1 to VRR3. J_{MMx} / J_{wi} VRR1-3: model mixture
 473 flux normalization with initial membrane water flux. MMx: x represents the composition of the model mixtures,
 474 P for proteins and L for lipids. Maximum SD 8% (Apostrophe mark ' and '' means repetition of the experiment)

475

476 *Table 5. Hydraulic performance of the membrane before during and after DF at 30 °C and 0.5bar **

N° Assay for DF	J_{wi} $L \cdot h^{-1} \cdot m^{-2}$	J_{wf} $L \cdot h^{-1} \cdot m^{-2}$	J_{MMx} VRR1-3 $L \cdot h^{-1} \cdot m^{-2}$	J_{MMx} / J_{wi} VRR1-3
MM _L γ_1	59	53	85-70	1.3-1.5
MM _L γ_1 '	44	42	78-67	1.5-1.6
MM _P γ_1	54	39	63-48	1.0-1.2
MM _P γ_1 '	19	19	28-27	1.4-1.5
MM _{P/L} γ_1	34	24	52-46	1.0-1.4
MM _{P/L} γ_1 '	38	27	61-49	1.3-1.5
MM _{P/L} γ_2	55	41	83-78	1.4-1.6
MM _{P/L} γ_2 '	20	16	27-21	1.3-1.5
RM γ_1	66	48	(0.3bar) 44-43	1.0-1.2

477 * J_W Membrane water flux before (i) or after (f) concentration experience. J_{MMx} VRR1-3: membrane model mixture
 478 flux variation during concentration, values calculated from VRR1 to VRR3. J_{MMx} / J_{wi} VRR1-3: model mixture

479 flux normalization with initial membrane water flux. MM_x: x represents the composition of the model mixtures,
480 P for proteins and L for lipids. Maximum SD 8%. (Apostrophe mark ' and '' means repetition of experiment)

481

482 3.2.2. *The interaction between lipids and proteins impacts the fouling*

483 Based on the resistance-in-series model, after a water flush, the resistance to water
484 permeation can be estimated as the sum of the clean membrane resistance, the resistance of the
485 physically reversible fouling, the resistance of the chemically reversible fouling, and the
486 resistance of the irreversible fouling [31,32]. Here, the membrane was rinsed with water to
487 remove the physically reversible fouling, thus the fouling resistance R_f represents the total
488 resistance of both chemically reversible fouling and irreversible fouling. It was calculated using
489 the initial water flux and the final water flux after water rinsing, for the coupons with the highest
490 initial water flux ($>30 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$) to facilitate comparison. The results are shown in Figure 4.
491 Besides, the lipids accumulated on the membrane after the concentration and water rinsing were
492 quantified by ATR-FTIR analysis (Figure 5).

493 The impact of the composition of the mixture on the process can be studied by
494 comparing the results of MM_P, MM_L and MM_{P/L} in CF and DF under the same hydrodynamic
495 condition γ_1 . The fouling resistance (Figure 4) is higher with proteins (MM_P) than with lipids
496 (MM_L), and this difference is more evident for DF. The proteins seem more likely to generate
497 a fouling, more stable at high shear rate than lipids. MM_P contains protein aggregates and
498 probably free proteins and it can be supposed that both can generate the membrane fouling,
499 with a possible reorganization at the membrane interface.

500 In the case of MM_{P/L}, containing both proteins and lipids, the fouling resistance (figure
501 4) is higher than with the separated compounds in CF and DF, but it is not the sum of the
502 resistances of lipids alone and proteins alone. A different structure of the fouling occurred. The
503 results of the particle size distribution analysis (Figure 2) in MM_{P/L} suggested a strong
504 reorganization of the proteins, which probably adsorbed at the droplets interface. Thus the
505 droplet interface was modified by proteins. According to the results of ATR-FTIR analysis in
506 Figure 5, comparing MM_L and MM_{P/L} in DF, the addition of proteins in the O/W system led to
507 an increased lipids accumulation on the membrane surface. Thus the presence of proteins
508 modified the behavior of lipids at the membrane surface. They facilitated their accumulation on
509 the membrane and induced a higher resistance. In the case of CF: the quantity of accumulated
510 lipids is the same order of magnitude with or without proteins, but the resistance is different,
511 thus the fouling structure is also different. this array of results leads to the hypothesis that

512 proteins interacted with lipids, induced a stronger adsorption of lipids on the membrane (the
513 lipids can not be removed by dynamic filtration when proteins are present) and that it had a
514 negative impact on the permeability of the membrane filtration (higher fouling resistance).

515

516 3.2.3. *The role of the shear rate on the fouling mitigation*

517 Comparing the CF and DF γ_1 resistance, it appears that with MM_L , R_f in CF is higher
518 than R_f in DF, showing that the higher shear rate is useful to eliminate the lipid fouling.

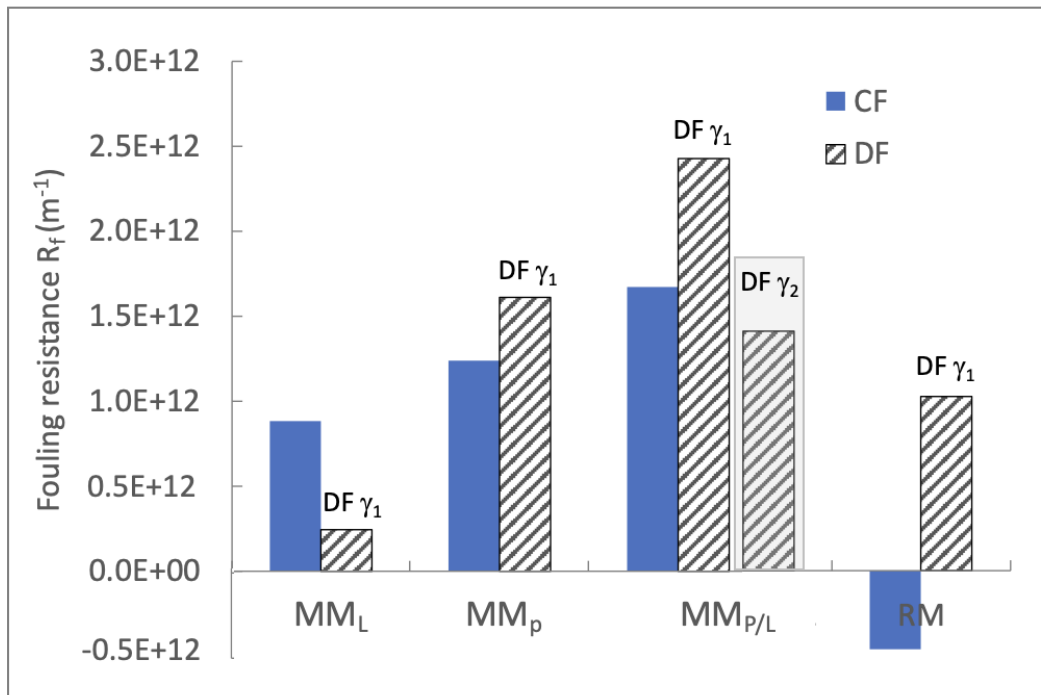
519 In presence of proteins, with MM_P and $MM_{P/L}$, the fouling resistance becomes higher in
520 DF than in CF. The shear rate may induce a different structure of the fouling (enhanced internal
521 fouling or denser fouling for example) leading to a lower permeability. Concerning the impact
522 of hydrodynamic conditions γ_2 on filtration performances, the fouling resistance (Figure 4) R_f
523 of $MM_{P/L}$ DF γ_2 is almost twice lower than $MM_{P/L}$ γ_1 and R_f of $MM_{P/L}$ CF is in between. The
524 hydrodynamic has two different impacts: the potential removal of the fouling but also the
525 modification of its structure, leading to a positive or negative effect on the permeation
526 depending on the filtrated product. The results of ATR-FTIR analysis also confirmed that the
527 lipid accumulation on the membrane surface for $MM_{P/L}$ DF γ_1 (analysis at medium position:
528 $400 \mu\text{g}\cdot\text{cm}^{-2}$) was much higher than in CF ($170 \mu\text{g}\cdot\text{cm}^{-2}$) and DF γ_2 (analysis at medium position:
529 $40 \mu\text{g}\cdot\text{cm}^{-2}$)).

530 The role of hydrodynamics is also highlighted for each DF experiment in figure 5. The
531 rotation of the disc creates a shear rate stronger on the periphery of the membrane than near the
532 center, which moves the lipids fouling deposit more efficiently from the periphery than from
533 the center of the membrane. Goh et al., 2018; Zhang et al., 2015 and Zhang and Ding, 2015
534 [52–54] also confirmed that shear-enhanced dynamic filtration effectively prevented the
535 deposition of substances on the membrane surface.

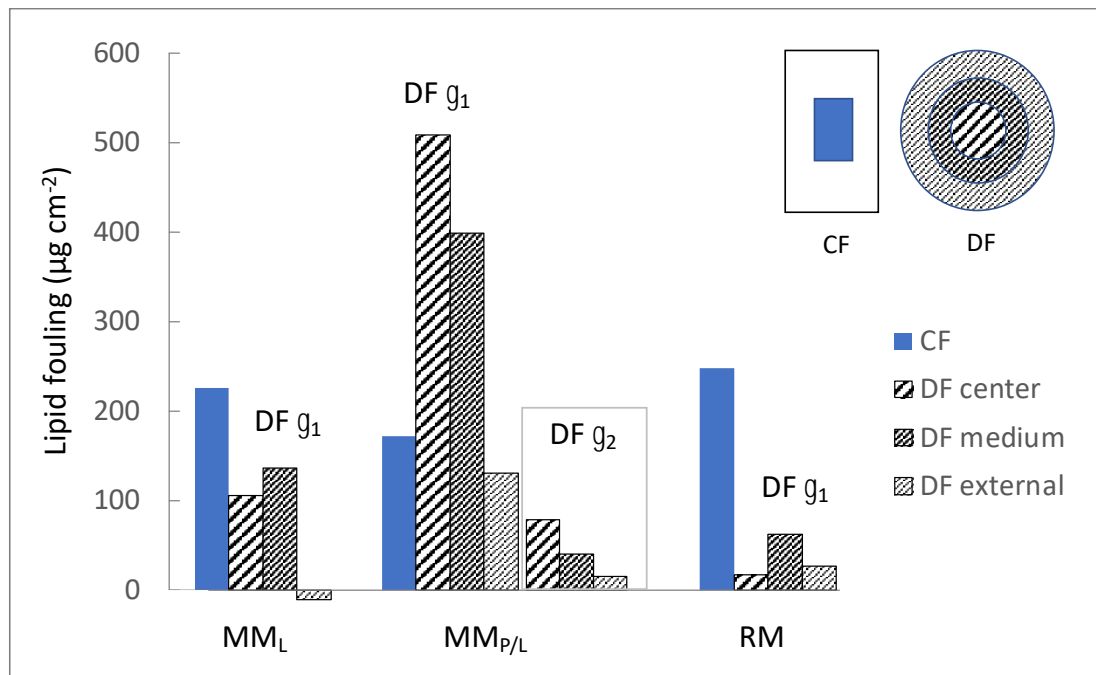
536 Based on these results the hypothesis is that at low shear rate (CF), the fouling can settle
537 but has a limited impact on the flux, thanks to an open structure. When the shear rate is higher
538 (DF γ_1), a different fouling organizes, which can be strongly lowered if the shear rate is high
539 enough (DF γ_2).

540 Comparing RM filtration in CF and DF, results are different. In CF, the filtration flux is
541 similar to the $MM_{P/L}$ but fouling resistance was negative even if lipids accumulated in the same

542 range ($250 \mu\text{g}\cdot\text{cm}^{-2}$). The hydrophilization of the membrane offsets the fouling impact and the
 543 hydrophilic molecules were not removed by water rinsing. This may be due to carbohydrates
 544 present in RM. In DF, the flux with RM is lower than with $\text{MM}_{\text{P/L}}$, but after rinsing, the fouling
 545 resistance is smaller and the quantity of lipids the same order of magnitude. It means that the
 546 physically reversible fouling is preponderant in this case.



547
 548 *Figure 4. Fouling resistance (R_f) of different mixtures in CF and DF under two hydrodynamic conditions*
 549 *(γ_1 and γ_2 – highlighted in light gray)*



550
 551 *Figure 5. Lipids membrane fouling characterization by ATR-FTIR analysis for model (MM) and real (RM)*
 552 *mixtures. The lipids were quantified in the center of the cross-flow membrane (CF) and in the center,*
 553 *intermediate and external annular bands of the dynamic filtration membrane (DF), for two different shear rates*
 554 *(γ_1 at $16\,000\text{ s}^{-1}$ and γ_2 $66\,000\text{ s}^{-1}$ -highlighted in light gray).*

556 3.2.4. Membrane selectivity

557 Table 6 presents the retention rates of total dry matter (DM), proteins and lipids. Firstly,
 558 we observe a total lipid retention for both model and real mixtures, which means the
 559 concentration of lipids from microalgae extract by membrane process is efficient. For proteins,
 560 different retention rates were observed.

561 During the filtration of MM_P, 57-77 % of protein retention rate were obtained in CF and
 562 DF. An important part of proteins aggregated as shown in Figure 2, but 23 – 41 % of proteins
 563 were probably free and passed through the membrane with a MWCO 0.1 µm. The free proteins
 564 were not visible in the volume size distribution due to the presence of large aggregates. But the
 565 Native-PAGE electrophoresis results (Figure 3) show that proteins smaller than 240 kDa could
 566 pass through the membrane (MM_P-P). According to SDS-PAGE electrophoresis, they contain
 567 mostly subunits below 50 kDa but unfortunately this information did not allow any protein
 568 identification.

569 In the presence of lipids (MM_{P/L} filtration), the retention rate of proteins increased to
 570 78-84% in CF and DF, whereas the aggregates characterized were smaller. This means that a
 571 part of the soluble proteins, which should permeate through the membrane, were retained at the

572 membrane interface and were not free anymore. The fouling contained more lipids than MM_L
573 (Figure 5) but also probably more proteins.

574 Besides, according to Courthaudon et al., 1991.; Damodaran, 2005 and Euston et al.,
575 1995 [21,43,44], when lecithin and protein-based emulsifiers are mixed before emulsification
576 process, those molecules compete with each other for the adsorption on the oil-water interface
577 and form multi-emulsifier micelles. This competitive adsorption can vary with the ratio of two
578 emulsifiers, and in our case, for $MM_{P/L}$, the amount of proteins-based emulsifier is much more
579 important than lecithin. Dickinson, 2001 [55] reported that the protein conformations around
580 the oil droplet are generally more stable than that of a small surfactant. In particular, the
581 sulfhydryl and disulfide groups in proteins make them irreversibly adsorbed to the oil-water
582 interface, creating a highly viscoelastic film. This may be the reason of the increased protein
583 retention rate in $MM_{P/L}$ and RM.

584 Accordingly, for real mixture filtration, it is interesting to compare our results with those
585 of Kulkarni and Nikolov, 2018 and Ursu et al., 2014 [17,19]. All of them filtered the supernatant
586 from *Chlorella vulgaris* after cell disruption by HPH and clarification, with a membrane PES
587 300 kDa using tangential filtration, under neutral pH conditions, similar protein retention was
588 reported (80-87 %). We could have expected more protein transmission with the larger MWCO
589 used in this work but it was not the case.

590 Comparing the retention rate of proteins and lipids (RR_{proteins} and RR_{lipids}) between
591 $MM_{P/L} \gamma_1$ and $RM \gamma_1$, no significant difference was observed, the difference in retention of DM
592 is due to the presence of a large amount of carbohydrates in RM ($0.72 \text{ g}\cdot\text{L}^{-1}$).

593 Regarding the effect of hydrodynamic conditions on the membrane selectivity,
594 comparing $MM_{P/L} \gamma_1$ and $MM_{P/L} \gamma_2$, no significant difference in retention rates (proteins and
595 lipids) was observed. Thus, these results confirm that higher shear force favors an increase in
596 flux and therefore productivity, but does not increase membrane selectivity. The aggregates
597 containing lipids and proteins seem very stable and large enough to be retained.

598
599

Table 6. Dry matter (DM), proteins and lipids retention rate (RR) at VRR3 for different model mixtures and real mixture

N° Assay	CF			DF		
	RR _{DM}	RR _{proteins}	RR _{lipids}	RR _{DM}	RR _{Proteins}	RR _{Lipids}
	%	%	%	%	%	%
MM _L γ_1	/	/	100	89.6±3.9	/	100
MM _L γ_1'	87.8±5.0	/	100	83.3±8.5	/	100
MM _P γ_1	44.4±6.6	/	/	31.7±1.2	58.8±3.0	/
MM _P γ_1'	22.8±0.4	57.0±1.6	/	55.9±1.3	77.0±1.2	/
MM _{P/L} γ_1	85.3±1.9	82.3±2.1	100	78.4±1.2	84.7±2.7	100
MM _{P/L} γ_1'	80.7±5.9	77.9±1.4	100	79.7±1.2	80.5±0.9	100
MM _{P/L} γ_2	78.3±2.4	74.5±3.3	100	82.3±6.5	81.0±4.1	100
MM _{P/L} γ_2'	/	/	/	85.7±0.8	92.3±2.9	100
RM γ_1	66.5±6.6	84.5±0.8	100	63.2±6.5	86.0±1.2	100

600 * MMx: x represents the composition of the model mixtures, P for proteins and L for lipids. (Apostrophe mark '
601 and '' means repetition of experiment)

602 4. Conclusion

603 The microalgae biorefining necessitates the coupling of cell disruption and separation
604 operation units. The intrinsic microalgae composition or the cell disruption was supposed to
605 generate interactions between biomolecules that can hinder the separation. The objective in this
606 work was to evaluate more specifically the impact of the lipids and proteins interaction on their
607 separation by microfiltration. Through the formulation of three representative model mixtures
608 containing lipids, proteins or both kinds of molecules, their filtration and comparison with real
609 microalgae extracts, it was demonstrated that the strong interaction between the biomolecules
610 in solution had a deep impact on filtration performances: the membrane fouling worsens and
611 the productivity and selectivity are limited. The dynamic filtration was tested to enhance the
612 performances compared to cross-flow filtration. The higher shear rate favored an increase in
613 flux and therefore productivity, but did not modify membrane selectivity. The concentration of
614 lipids was possible, with a total retention of lipids, but their interaction with proteins induced a
615 higher fouling, and limited the protein permeation. The results obtained with the lipids and
616 proteins mixture were similar to the real mixtures thus bringing a part of understanding on the
617 limitation met with microalgae extracts. It was therefore demonstrated that the interaction
618 between molecules drives the separation efficiency and should be mitigated before filtration in
619 future works to allow the whole biomass biorefining.

620

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627 Authorship contributions: E. Couallier, M. Frappart and S. Liu were responsible for the
628 conception and design of the study. The data were acquired by S. Liu, C. Rouquié and M.
629 Rabiller Baudry. The data were analyzed and interpreted and the manuscript written by E.
630 Couallier, S. Liu, M. Rabiller Baudry, A. Szymczyk C. Rouquié and M. Frappart. All the
631 authors approved the final manuscript.

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