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**Reference:**

Fret J., Roef L., Diels Ludo, Tavernier Serge, Vyverman W., Michiels M.- Implementation of flocculation and sand filtration in medium recirculation in a closed microalgae production system

Algal Research - ISSN 2211-9264 - 13(2016), p. 116-125

Full text (Publisher's DOI): <http://dx.doi.org/doi:10.1016/J.ALGAL.2015.11.016>

To cite this reference: <http://hdl.handle.net/10067/1300330151162165141>

1 Title: Implementation of flocculation and sand filtration in medium recirculation in a  
2 closed microalgae production system

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9 **Abstract**

10 Recycling growth medium is a necessity to reduce production cost and ecological foot  
11 print of large scale microalgae production systems. To prevent contamination and/or  
12 enrichment with particulate matter, medium recycling requires pre-treatment of the  
13 centrifuge supernatant (centrate), prior to medium replenishment and re-use. In this  
14 study, we investigated the applicability of high pH induced flocculation and/or sand  
15 filtration to interface with an existing microfiltration setup in order to prepare recycled  
16 growth medium for the mass cultivation of marine microalgae. Sand filtration partly  
17 alleviated the burden on subsequent microfiltration, but proved to be particularly  
18 useful to remove high pH induced flocs from the centrate. Combination of both  
19 techniques resulted in a removal of  $78 \pm 18\%$  of particles, resulting in an improvement  
20 of 'modified fouling indices' by  $75 \pm 19\%$ . Despite a partial to complete removal of  
21 remaining nutrients such as phosphate, calcium and magnesium during treatment, a  
22 cost saving of 72% compared to a scenario with fresh medium can be achieved.

23 Highlights

- 24 - Characterisation of flocculation and sand filtration of centrifuge supernatant
- 25 - Optimal combination of flocculation and sand filtration was established
- 26 - Pre-treatments enable sustainable recirculation of spent microalgae growth medium
- 27 *Keywords:* Microalgae, flocculation, sand filtration, medium recycling

28 **1. Introduction**

29 In order to tap into the enormous potential of microalgae as resource for bulk biofuel,  
30 feed/food and chemical applications, substantial reduction in production cost is  
31 needed [1]. Recycling culture broth is key to further decrease these costs [2], especially  
32 in cases where final applications call for the use of more expensive feed or food grade  
33 ingredients. At present, centrifugation is the most commonly used method for  
34 harvesting microalgae. Due to the limited separation efficiency inherent to the  
35 technique, a fraction of particles present in culture broth, including intact and broken  
36 algae cells, bacteria, and other debris are retained in the centrifuge supernatant  
37 (centrate). While in principal, this centrate could be reused as growth medium after  
38 nutrient replenishment [3–5], applying non-treated centrate as culture medium will  
39 contaminate and/or eventually enrich the production system with unwanted algae,  
40 protozoan grazers, cell debris and bacteria under normal field operating conditions.  
41 Therefore a recycling scheme for culture medium calls for post-treatment of the  
42 centrate prior to medium replenishment and re-use. At present, no practical post-  
43 treatment process is available that can feed into a microfiltration unit such as the one  
44 that is currently operated by us to provide sterile media to Proviron's ProviAPT™  
45 production system. De Baerdemaeker et al. [6] reported that during microfiltration of  
46 centrate heavy fouling occurs which results in a severe loss of permeability. Dissolved  
47 algogenic organic matter (AOM) including polysaccharides, proteins and humic acid-  
48 like organics [7], are reported to cause substantial membrane fouling during  
49 microfiltration (MF) [8], ultrafiltration (UF) [9,10] and nanofiltration (NF) [11]. Pre-  
50 treatments prior to membrane filtration which decrease the concentration of both

51 suspended solids and dissolved AOM of the centrate are therefore needed to reduce  
52 the load on the membrane and thus prolong its lifespan.

53 Coagulation pre-treatment using metal salts, such as aluminium and iron is commonly  
54 applied to control fouling in MF or UF systems [12,13] by removing both particulate  
55 and dissolved materials [14]. For our purpose, high pH induced flocculation is an  
56 attractive alternative because it is low-cost and is low in energy consumption.

57 Furthermore, it is non-toxic [15], a prerequisite for further use of the cleared medium.

58 The technique was found effective for harvesting microalgae [16,17]. Both NaOH and  
59 hydrated lime,  $\text{Ca}(\text{OH})_2$ , are suitable to increase pH in a controlled manner [18].

60 Presence of AOM, however, interferes with the process [19], resulting in the need of a  
61 higher pH to induce flocculation [20]. In this study the applicability of high pH induced  
62 flocculation of centrate was investigated.

63 Sand filtration is frequently used as a cheap, easy and robust technique to remove  
64 suspended solids from water. The method has a broad application in the water  
65 treatment field, where it has proved its efficacy [21]. A few studies indicated the  
66 applicability of sand filtration to separate algae from the liquid broth [22,23]. However,  
67 the use of sand filtration as a pre-treatment technique to polish centrate has not been  
68 investigated previously. In this study the efficiency of sand filtration to eliminate  
69 suspended solids from the centrate prior to membrane filtration was researched.

70 Rapid sand filtration is usually preceded by coagulation, flocculation and/or  
71 sedimentation to increase its removal efficiency. Therefore, implementation of both  
72 techniques at optimum settings in a linked setup was also studied in order to assess

73 which (combination of) approaches can be used in a large scale installation to reduce  
74 fouling and increase MF performance (shorter filtration times, longer filter lifespan).

## 75 2. Materials and methods

### 76 2.1. Algae cultivation – centrate collection

77 The centrate used in this study was obtained from a *Nannochloropsis* sp. culture  
78 cultivated semi-continuously in a 12 m<sup>3</sup> module of an outdoor production system of  
79 the ProviAPT™ photobioreactor [24,25] (Fig 1).



80  
81 **Fig 1: View of a ProviAPT™ microalgae production plant of 48 m<sup>3</sup> culture volume**

82 The growth medium consisted of artificial seawater supplemented with nutrients in  
83 concentration ratios derived from the *f* formulation [26]. The seawater was prepared  
84 using a refined sea salt mixture (Zoutman Industries, Belgium) to obtain a salinity of 26  
85 g L<sup>-1</sup>. After addition of nutrients, the medium was filtered (0.2 μm, KrosFlo<sup>®</sup>,  
86 Spectrum<sup>®</sup>, USA) prior to pumping to the reactors. Algae were harvested daily and  
87 dewatered using an automated desludging disc centrifuge (SSD 18-06-007, GEA  
88 Westfalia, Germany), yielding an algae paste and a supernatant, the centrate. The

89 density of the harvests which provided the used media, ranged from 1.2 to 3.1 g L<sup>-1</sup>.  
90 These centrates were, in turn, characterized by dry weight and optical density. In  
91 addition, macronutrient concentrations of the centrates were determined.  
92 Magnesium, calcium and trace elements were only measured of selected centrates  
93 (2.5 Analysis).

#### 94 2.2. General setup of flocculation experiments

95 High pH induced flocculation was investigated using conventional jar test experiments  
96 [27]. Centrate was divided in 200 mL portions in 1 L beakers and mixed using a  
97 magnetic stirrer. The pH was adjusted to five different levels ranging from 9.5 up to  
98 11.5 with a 0.5 interval by addition of 1 M NaOH or Ca(OH)<sub>2</sub>. The amount of alkali  
99 needed to obtain the desired pH was recorded. Samples were stirred at 300 rpm for 10  
100 minutes following pH adjustment. Next, solutions were allowed to settle for 60  
101 minutes after which a 1 mL sample was collected from the centre of the clarified zone.  
102 The flocculation efficiency  $\eta_a$  was determined based on the decrease in optical density  
103 of the cleared centrate as suggested by Vandamme et al. [27]:

$$104 \quad \eta_a = \frac{OD_i - OD_f}{OD_i} \quad (1)$$

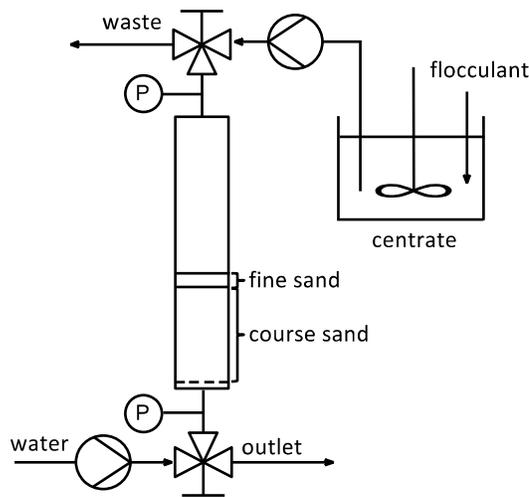
105 where OD<sub>i</sub> is the optical density of the solution after sedimentation without pH  
106 adjustment (control), and OD<sub>f</sub> is the optical density of the suspension after flocculation  
107 and settlement. Next, a 15 mL sample was taken from the cleared upper part of the  
108 solutions to investigate nitrate and phosphate removal. Remaining quantities were  
109 then stirred again and equivalent samples were taken from the blended mixtures. Both  
110 types of samples were neutralised with 2 M HCl and the amounts of acid required to

111 reach their initial pH values were recorded. To assess improvement of filterability, the  
112 modified fouling index (MFI) was determined of selected samples from the upper part  
113 of flocculated solutions and of blended mixtures. At the end of the flocculation  
114 experiment, the morphology of settled flocs was documented using a BX51 Olympus  
115 microscope with differential interference contrast (DIC) fitted with an E410 Olympus  
116 camera.

117 Furthermore, calcium and magnesium removal from representative samples was  
118 calculated. The removal of trace elements due to flocculation was also investigated to  
119 determine the need for replenishment prior to reusing the medium. To this end,  
120 samples were taken from the top layer of NaOH flocculated centrate and, after  
121 neutralisation, elemental composition was determined. A chemical equilibrium model  
122 (Visual MINTEQ 3.0 [28]) was used to predict the theoretical manifestation and  
123 speciation of precipitates in standard medium with pH increase.

### 124 *2.3. General setup of sand filtration experiments*

125 The sand filter was constructed of a polyvinyl chloride column of 1 m with inner  
126 diameter of 45.2 mm. At the top and the bottom of the column a 3-way ball valve was  
127 installed to allow performing a backflush rinsing. After the top and prior to the bottom  
128 valve, a gauge was fixed to observe pressure increase (Fig 2).



129

130 **Fig 2: Experimental setup of the sand filtration unit.**

131 Sand with two different grain sizes was used to fill the column. 'Coarse' sand had a  
 132 diameter between 250 and 500  $\mu\text{m}$ , while 'fine' sand had a median particle size ( $D_{v50}$ )  
 133 of 170  $\mu\text{m}$ . Sabiri et al. [22] suggested a bed height of 40 cm to obtain a stable removal  
 134 efficiency. In this study, the bed was packed with 35 cm of coarse sand and 5 cm of  
 135 fine sand on top to improve separation efficiency. Preliminary experiments indicated  
 136 that a higher sand bed of fine sand resulted in rapid blockage of the filter, decreasing  
 137 the flow drastically.

138 Before filtration, the bed was completely fluidized by backwashing with water to  
 139 remove air bubbles from the system and to allow sand particles to redistribute  
 140 according to velocity. Next, particles settled by sedimentation with the finer particles  
 141 located in the upper section of the bed while larger ones were found at the bottom.  
 142 Remaining water was evacuated through the bottom valve, resulting in a slight  
 143 compaction of the sand bed. During filtration, the water flowed in a downward  
 144 direction through the fixed bed using a demand spray pump (RLF122202, Flojet). The

145 inlet flow was regulated using an adjustable power supply and voltage was maintained  
146 constant during filtration. The increase in height of the water column above the sand  
147 bed was tracked throughout. At the outlet 50 mL samples were taken every five  
148 minutes for further analysis. Between the five minute intervals, the exhaust flow rate  
149 was determined. Afterwards, optical density of the permeate samples was measured  
150 and filtration efficiency calculated, similar to the flocculation efficiency. Next, as  
151 recycling of permeate after sand filtration is the final goal, nitrate and phosphate  
152 concentrations were measured to evaluate their retention. Calcium and magnesium  
153 were also measured before and after sand filtration. Furthermore, MFI's of selected  
154 samples were compared to the control to assess improvement of filterability. After  
155 each filtration run, the sand bed was backflushed with tap water until the overflow  
156 was clear.

#### 157 *2.4. Centrate clearing by means of combined flocculation – sand filtration*

158 To improve the separation efficiency of the sand filtration, flocculation of the centrate  
159 was implemented as a pre-treatment. Both NaOH and Ca(OH)<sub>2</sub> were used to alter the  
160 pH of 15 L centrate portions to 10.5 and 11.5 prior to sand filtration. After pH  
161 adjustment, centrates were mixed on a magnetic stirrer (1200 rpm) for 10 minutes to  
162 allow aggregates to form. During filtration over the sand bed, the inlet solution was  
163 continuously stirred gently to provide a homogenous mixture (Fig 2). Filtration,  
164 sampling and analysis were performed similar to the sand filtration experiments. After  
165 filtration of flocculated centrate, the bed was backflushed with neutral or acidified  
166 water (pH 2.2).

167        *2.5. Analysis of centrate composition*

168        Centrate was characterised by the amount of suspended solids, which were  
169        determined gravimetrically. Aliquots of 20 mL centrate were filtered through pre-  
170        weighed, pre-dried A/C glass-fiber filters (pore size 1 µm, Pall, USA). Subsequently,  
171        filters were rinsed with an equal amount of 0.5 M ammonium formate to remove  
172        interfering salts. Next, the filters were dried overnight at 110 °C until constant weight  
173        to calculate the dry weight.

174        Flocculation and sand filtration efficiency was assessed based on optical density  
175        measured as the absorbance at 550 nm in a UV-VIS spectrophotometer (Cary 50 Conc,  
176        Agilent Technologies).

177        Nutrient concentrations were determined following the procedures described in the  
178        Standard Methods [29] after filtration of the samples (0.2 µm). The analysis of nitrate  
179        is based on the absorbance at 220 and 275 nm in an acid environment ([29], method  
180        No. 4500 B Nitrate). The ascorbic acid method was applied to determine the amount of  
181        reactive phosphates ([29], method No. 4500-P E). Meanwhile, determination of salt  
182        cations (Mg and Ca) was conducted with an atomic absorption spectrophotometer  
183        (AAS), while quantification of trace element concentrations was conducted with a  
184        high-resolution inductively-coupled plasma mass-spectrophotometer (HR-ICP-MS).

185        *2.5.1. Modified fouling index (MFI) assessment*

186        The modified fouling index (MFI) was used as a tool to assess the fouling potential of  
187        the centrate. The MFI is determined by a constant-pressure dead-end filtration process

188 and was originally defined as an index of the fouling potential of reversed osmosis feed  
189 water by Schippers and Verdouw [30]. The time required to filter a fixed volume of 500  
190 mL through a standard 0.45  $\mu\text{m}$  microfiltration membrane with a diameter of 47 mm  
191 (PES Supor<sup>®</sup>-450, Pall, USA) under constant pressure (200 kPa) dead-end filtration  
192 mode is registered. The samples were diluted 200 times in reversed osmosis water of  
193 which the pH was adjusted corresponding to the pH of the sample. The cumulative  
194 permeate volume and filtration time is recorded. Next, the obtained data were fit in  
195 commonly used models to analyse the membrane filtration process [31], described by  
196 equation (2)-(5).

$$197 \quad \frac{d(V)}{dt} = J_0 - k_b \cdot V \quad (2)$$

$$198 \quad \frac{t}{V} = \frac{1}{J_0} + \frac{k_s}{2} t \quad (3)$$

$$199 \quad \frac{dt}{d(V)} = \frac{1}{J_0} + k_i t \quad (4)$$

$$200 \quad \frac{t}{V} = \frac{1}{J_0} + \frac{k_c}{2} \cdot V \quad (5)$$

201

202 In these equations,  $t$  is filtration time,  $V$  is the cumulative permeate volume,  $J_0$  is the  
203 initial flux, while  $k_b$ ,  $k_s$ ,  $k_i$  and  $k_c$  (i.e., the above mentioned MFI) are the coefficients of  
204 complete blocking, standard blocking, intermediate blocking, and cake filtration  
205 models respectively. Modified fouling indices were derived from the model which  
206 showed the best fit and used to compare the filterability of the samples. By  
207 distinguishing which model showed the best fit, additional information about the  
208 reigning filtration modus was obtained.

### 209 **3. Results and discussion**

210 3.1. Centrate clean-up by flocculation

211 3.1.1. Efficiency of NaOH and Ca(OH)<sub>2</sub> promoted flocculation of centrate

212 The applicability of flocculation of exhaust medium was investigated using three  
213 different centrates (Table 1).

214 **Table 1. Characteristics of three centrates used to test flocculation. Measurements**  
215 **were performed in duplo (n = 2).**

Centrate number	Dry weight particulate matter (g L <sup>-1</sup> )	P concentration (μM)	N concentration (mM)	OD (Abs 550nm)
1	0.27 ± 0.03	2.1 ± 0.1	0.42 ± 0.04	0.84 ± 0.02
2	0.23 ± 0.08	59 ± 2	1.29 ± 0.08	0.63 ± 0.02
3	0.15 ± 0.04	706 ± 14	2.56 ± 0.03	0.48 ± 0.02
Fresh medium	-	1700	25	-

216 Both flocculation efficiency and filterability, determined as MFI's, increased

217 significantly with increasing pH (ANOVA, P<0.01), as illustrated in Fig 3 and

218 Table 2 respectively. Hence, augmentation of pH with either NaOH or Ca(OH)<sub>2</sub> can be

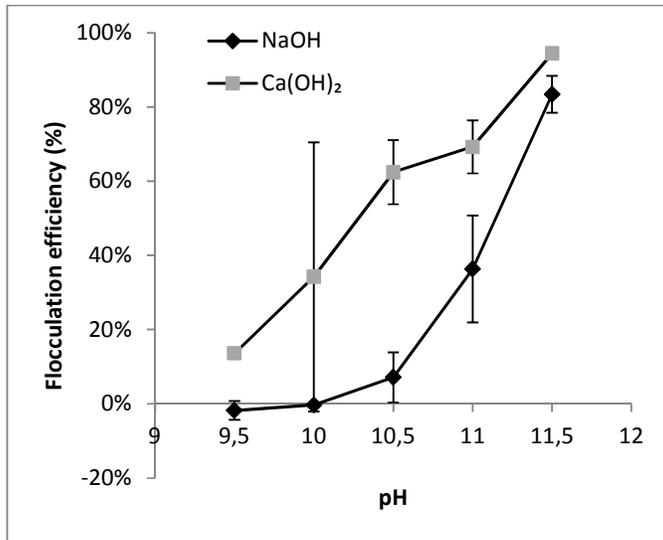
219 applied in order to flocculate centrate and improve its filterability. Acquired filtration

220 data fitted best with the standard blocking and cake filtration model [31] (

221 Table 2). Merely based on flocculation efficiency and improvement of filterability, one

222 would be inclined to use Ca(OH)<sub>2</sub> flocculation at highest pH of 11.5 to flocculate

223 centrate.



224

225 **Fig 3: Flocculation efficiency versus pH for two different alkali species. Centrates 1**  
 226 **and 2 were investigated.**

227 **Table 2: MFI's ks and kc of flocculated centrate (n=2) using two different alkali**  
 228 **species on centrates 1 and 2.**

Alkali	pH	ks (m <sup>-1</sup> )	kc (s·m <sup>-2</sup> )
NaOH	control	1.92 ± 0.03	9E+03 ± 1E+03
	9.5	1.91 ± 0.02	7E+03 ± 4E+03
	10.5	1.84 ± 0.06	3E+03 ± 2E+03
	11.5	0.21 ± 0.04	17 ± 4
Ca(OH) <sub>2</sub>	control	1.91 ± 0.02	5E+03 ± 3E+02
	9.5	1.88 ± 0.02	6E+03 ± 1E+03
	10.5	1.6 ± 0.4	3E+3 ± 4E+03
	11.5	0.3 ± 0.1	3E+01 ± 2E+01

229

230 The MFI's of stirred flocculated solutions were also measured. Due to the aggregation

231 of suspended particles, the filtration mechanism changed from standard blocking

232 towards cake filtration. The ks and kc indices of centrates flocculated at pH of 11.5 (ks

233 of  $5.0 \pm 0.2$  and kc of  $9 \cdot 10^3 \pm 5 \cdot 10^3$ ) were significantly lower compared to control

234 indices (ks of  $6.5 \pm 0.4$  and kc of  $4 \cdot 10^4 \pm 2 \cdot 10^4$ ) (ANOVA, P<0.01). Thus, filterability of

235 centrate flocculated at high pH was improved even without removal of flocculated  
236 particles.

237 These results suggest the feasibility of flocculation to improve filterability of the  
238 centrate. However, flocculation at intermediate alkaline pH did not yield consistent  
239 efficiencies when centrate features changed. Furthermore, divergent efficiencies were  
240 obtained when different alkalis were used.

#### 241 *3.1.2. Implication of NaOH or Ca(OH)<sub>2</sub> utilization*

242 A pH increase induced by addition of Ca(OH)<sub>2</sub> resulted in significantly higher  
243 flocculation efficiencies (ANOVA, P<0.01) compared to NaOH flocculation (Fig 3 and  
244 Table 3). This effect was pronounced at slightly to moderately alkaline pH's, where  
245 calcium phosphate precipitation is known to induce flocculation [20]. On the one hand,  
246 Ca(OH)<sub>2</sub> flocculation caused a higher phosphate removal at slightly to moderately  
247 alkaline pH values (Table 3). This result suggests that Ca(OH)<sub>2</sub> flocculation caused  
248 higher calcium phosphate precipitation compared to NaOH flocculation, inducing  
249 better removal of organic substances.

250 Similar results were obtained by Castrillo et al. [32] when comparing the use of  
251 Ca(OH)<sub>2</sub> and NaOH to flocculate microalgae. Yet, these authors also noted the  
252 formation of calcium carbonate precipitates with Ca(OH)<sub>2</sub> flocculation which ended up  
253 in the flocculated pellet [32]. Similarly, microscopic analysis demonstrated the  
254 appearance of rather large calcium carbonate crystals in the flocs at slightly to  
255 moderately alkaline pH values (Fig 4). The formation of calcium carbonate crystals is  
256 likewise suggested by a much higher calcium removal in relation to phosphate

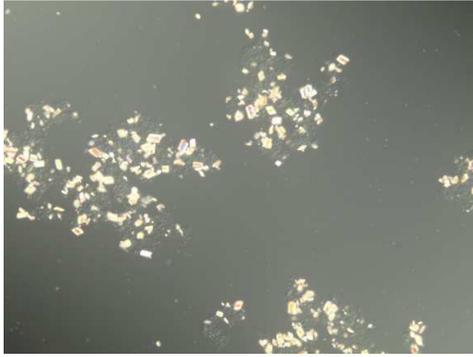
257 compared to the 5:3 M Ca:P stoichiometry of hydroxyapatite ( $\text{Ca}_5(\text{PO}_4)_3$ ) which was  
 258 believed to precipitate during flocculation (modelled with Visual MINTEQ, [33,34]).  
 259 These crystals can enhance sweep flocculation, contributing to the higher removal  
 260 rates obtained by  $\text{Ca}(\text{OH})_2$  flocculation. Since removed phosphate concentrations are  
 261 three orders of magnitude smaller than the amounts of removed calcium, precipitation  
 262 of calcium carbonate is likely to have a much higher influence on the flocculation  
 263 efficiency at slightly to moderately alkaline pH values. A higher  $\text{Ca}(\text{OH})_2$  requirement  
 264 to raise the pH to equivalent values compared to NaOH (see section 3.1.3), however,  
 265 diminishes the advantage of the higher efficiencies achieved.

266 **Table 3. Influence of alkali species, phosphate concentration and pH on flocculation**  
 267 **efficiency, calcium removal and phosphate removal. Removals are expressed as**  
 268 **changes in molar concentration. Calcium removal is calculated as the difference**  
 269 **between the remaining dissolved calcium after flocculation and the initial**  
 270 **concentration supplemented with the added  $\text{Ca}(\text{OH})_2$  (if applied).**

271

Alkali	Phosphate start concentration ( $\mu\text{M}$ )	Calcium start concentration (mM)	pH	Flocculation efficiency (%)	Remaining calcium (mM)	Remaining phosphate ( $\mu\text{M}$ )
NaOH	$3 \pm 1$	$1.97 \pm 0.06$	9.5	$2 \pm 2$	$1.1 \pm 0.2$	$2 \pm 2$
			10.5	$10 \pm 8$	$0.32 \pm 0.02$	$2.5 \pm 0.5$
			11.5	$89 \pm 2$	$0.19 \pm 0.06$	$0.2 \pm 0.2$
	$364 \pm 14$	$1.97 \pm 0.06$	9.5	$10 \pm 3$	$1.5 \pm 0.1$	$193 \pm 27$
			10.5	$40 \pm 3$	$0.39 \pm 0.05$	$28 \pm 20$
			11.5	$90 \pm 1$	$0.23 \pm 0.06$	$3 \pm 3$
$\text{Ca}(\text{OH})_2$	$4 \pm 4$	$9.47 \pm 0.06$	9.5	$13 \pm 3$	$1.54 \pm 0.02$	$1 \pm 0.6$
			10.5	$43 \pm 14$	$0.8 \pm 0.1$	$0 \pm 0.1$
			11.5	$92 \pm 4$	$6.6 \pm 0.1$	$-0.06 \pm 0.06$
	$371 \pm 4$	$8.22 \pm 0.06$	9.5	$24 \pm 4$	$0.99 \pm 0.02$	$71 \pm 27$
			10.5	$64 \pm 5$	$0.92 \pm 0.03$	$4 \pm 3$
			11.5	$92 \pm 4$	$6.8 \pm 0.4$	$0.2 \pm 0.2$

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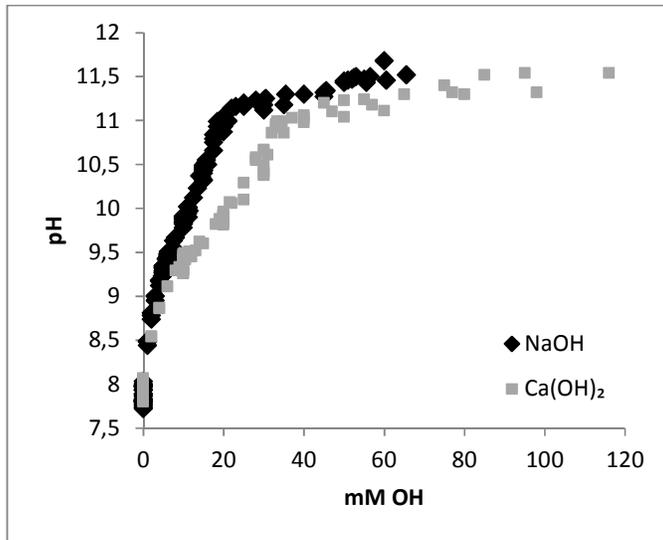


273

274 **Fig 4. DIC image of  $\text{Ca}(\text{OH})_2$  flocculated aggregates at pH 10 containing crystals**

275 *3.1.3. Chemicals consumption in response to pH increase*

276 Fig 5 shows the pH increase relative to the amount of added hydroxide. The obtained  
277 curves resemble titration curves of a weak acid with a strong base. Hence, to obtain a  
278 pH value above 11 disproportionately large amounts of alkali are required. Due to the  
279 lower dissociation constant of  $\text{Ca}(\text{OH})_2$ , a 1.6 times higher dosage on average is needed  
280 to obtain the same pH in comparison with NaOH. Depending on the chemical used,  
281 sodium or calcium ions were also added to the centrate. To obtain a pH of 10.5, 15.2  
282 mM sodium is added which is equivalent to 3.8% of the original medium  
283 concentration. However, 29.3 mM of calcium is added as  $\text{Ca}(\text{OH})_2$  to obtain a pH of  
284 10.5 which corresponds to 861% of the medium concentration. During the flocculation  
285 process, calcium phosphates and carbonates will precipitate and part of the excess  
286 calcium will disappear again from the medium.

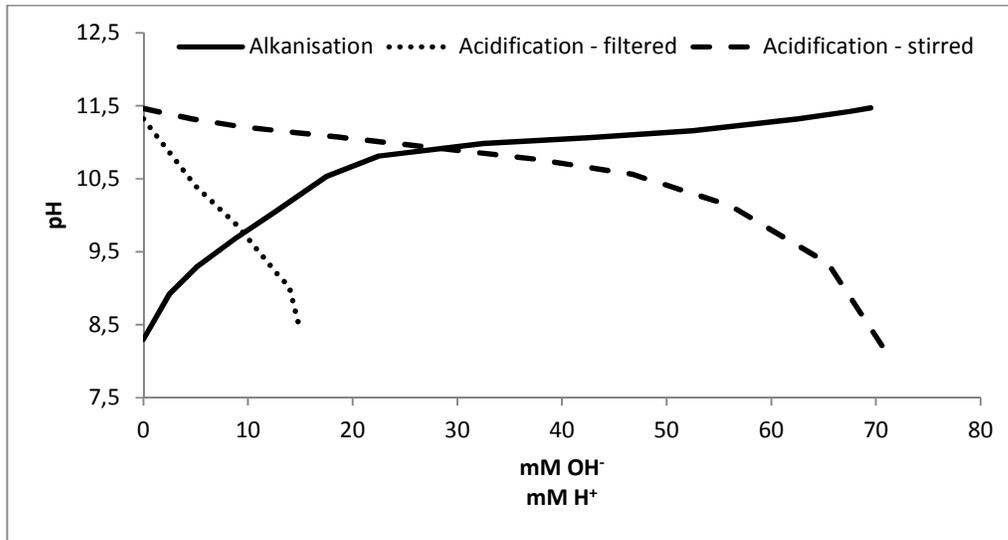


287

288 **Fig 5. Increase in pH as a function of hydroxide concentration added in the form of**  
 289 **NaOH or Ca(OH)<sub>2</sub>**

290 To enable recycling of the medium, cleared centrate requires neutralisation. Addition  
 291 of equimolar amounts of H<sup>+</sup> restores the pH value of stirred NaOH flocculated centrate  
 292 (Fig 6). Since OH<sup>-</sup> ions partake in the formation of crystals and flocs, they are removed  
 293 from the centrate after flocculation. Consequently, the H<sup>+</sup> requirement to neutralise  
 294 the cleared solution is reduced 4.8 times on average. Neutralisation with HCl adds a  
 295 maximum relative amount of salt of 14% to the standard concentration. This means  
 296 that even under the most thorough flocculation conditions a continuous recycle of  
 297 87.7% can be maintained without increase in salinity.

298



299

300 **Fig 6: OH<sup>-</sup> and H<sup>+</sup> requirement for respective alkanisation and subsequent**  
 301 **acidification of centrate that was either stirred or filtered upon flocculation. OH<sup>-</sup> was**  
 302 **added as NaOH and H<sup>+</sup> as HCl.**

303 *3.1.4. Influence of phosphate concentration on flocculation*

304 Flocculation of microalgae has been shown to depend on the presence of relatively  
 305 high concentrations of phosphate in the medium [20]. In addition, Bernhardt et al. [35]  
 306 showed that even low concentrations of AOM (a few mg C L<sup>-1</sup>) impaired the  
 307 flocculation process. When AOM is present, flocculation will only occur sufficiently if  
 308 phosphate concentrations exceed 350 μM and calcium concentrations exceed 500 μM  
 309 [20]. Due to the marine nature of the growth media used in this study, with a calcium  
 310 concentration of 3.4 mM, the latter condition is fulfilled. Phosphate, however, is a  
 311 macronutrient which is almost completely consumed during algae cultivation. Two out  
 312 of three tested centrates contained less than 350 μM of phosphate (Table 1).  
 313 Supplementation with KH<sub>2</sub>PO<sub>4</sub> up to 364 ± 14 μM phosphate resulted in significantly  
 314 higher flocculation efficiencies for NaOH flocculation (ANOVA, P<0.005, Table 3) and  
 315 an improved filterability of the centrate at moderately alkaline pH values. Since

316  $\text{Ca}(\text{OH})_2$  flocculation was already more effective at original phosphate concentrations -  
317 supposedly due to the additional formation of calcium carbonates - no significantly  
318 higher removal rates were reached ( $\alpha=0.05$ ) by this increase in phosphate  
319 concentration.

320 At a pH above 10.5, precipitation of magnesium hydroxides will occur and contribute  
321 to the removal of organic substances. Flocculation at high pH is less affected by the  
322 increase of phosphate due to the lower relative contribution of calcium phosphate  
323 precipitation at these pH values. Thus, adding surplus phosphate is unnecessary to  
324 obtain a sufficient flocculation at high pH.

325 Due to the variability of algae cultivation, phosphate concentration of obtained  
326 centrates may vary. One of the three centrates tested contained an initial phosphate  
327 concentration of 706  $\mu\text{M}$  and slightly less organic matter (Table 1). Despite the higher  
328 phosphate concentration, similar flocculation efficiencies were attained compared to  
329 flocculation of centrates with a phosphate concentration of 350  $\mu\text{M}$  (data not shown).  
330 Phosphate concentrations higher than 350 $\mu\text{M}$  will therefore not contribute to the  
331 flocculation efficiency. Moreover, due to the cost and the declining phosphate  
332 reserves, it is not considered sustainable to add additional phosphate to the medium  
333 to enhance flocculation. Beukels et al. [20] suggested re-dissolving all precipitated  
334 phosphate after flocculation by mild acidification. However, adding additional  
335 phosphate is most preferably avoided.

### 336 *3.1.5. Nutrient removal caused by alkali induced flocculation*

337 Nitrate and phosphate removal

338 As expected, nitrate was not removed from the medium as it does not participate in  
339 the flocculation process (overall removal of  $0.6 \pm 4.2\%$ ). Thus, residual nitrate can be  
340 reused entirely. Phosphates, on the other hand, do partake in the flocculation process  
341 as mentioned above. Phosphate removal depends on which alkali was used and on  
342 initial phosphate concentration (ANOVA,  $P < 0.05$ ).  $\text{Ca}(\text{OH})_2$  induced flocculation results  
343 in a significantly higher phosphate removal at the respective pH levels applied.  
344 Nevertheless, at pH 11.5 most of the phosphate ( $99 \pm 3\%$  on average) is removed,  
345 regardless of the phosphate start concentration, centrate and alkali. Hence, partial or  
346 complete replenishment of phosphate is necessary prior to medium reuse.

#### 347 Magnesium and calcium removal

348 As magnesium and calcium contribute to the flocculation process [17,36,37] one can  
349 expect them to be removed during the course of it. As displayed in Table 4, magnesium  
350 removal is significantly dependent on pH and on the alkali species applied (ANOVA,  
351  $P < 0.001$ ).  $\text{Ca}(\text{OH})_2$  flocculation results in a higher removal. At the concentrations tested  
352 phosphate hardly affects magnesium withdrawal. At high pH magnesium hydroxides  
353 form. When flocculation is executed at high pH, on average  $71 \pm 6\%$  (or  $28 \pm 2 \text{ mM}$ ) of  
354 the original magnesium content of fresh medium has to be replenished.

355 At each pH level, calcium removal was significantly dependent on the applied base  
356 (ANOVA,  $P < 0.005$ ). These removals, as summarized in Table 4, are calculated by  
357 comparing the remaining concentration with the total calcium concentration upon  
358 addition of the alkaline chemicals. Especially at a pH of 9.5, the difference in calcium  
359 removal between NaOH and  $\text{Ca}(\text{OH})_2$  flocculation is distinct. It can be attributed to the

360 calcium carbonate formation and higher flocculation efficiency attained with Ca(OH)<sub>2</sub>.  
 361 Similar to magnesium, when NaOH is used, on average 93.9 ± 0.8% (or 3.19 ± 0.03 mM)  
 362 of the original calcium will need to be replenished after flocculation at high pH prior to  
 363 recycling of the growth medium.

364 Use of Ca(OH)<sub>2</sub> can bring additional calcium to the medium. For instance, despite the  
 365 high calcium removal, surplus calcium (6.7 ± 0.2 mM) in comparison to the standard  
 366 medium concentration (3.4 mM) is present after flocculation at pH 11.5. Under these  
 367 conditions, calcium will accumulate in the medium during repeated medium reuse  
 368 cycles. At pH 9.5 and 10.5, calcium removal was sufficient to obtain calcium  
 369 concentrations which were slightly lower than the standard concentration.

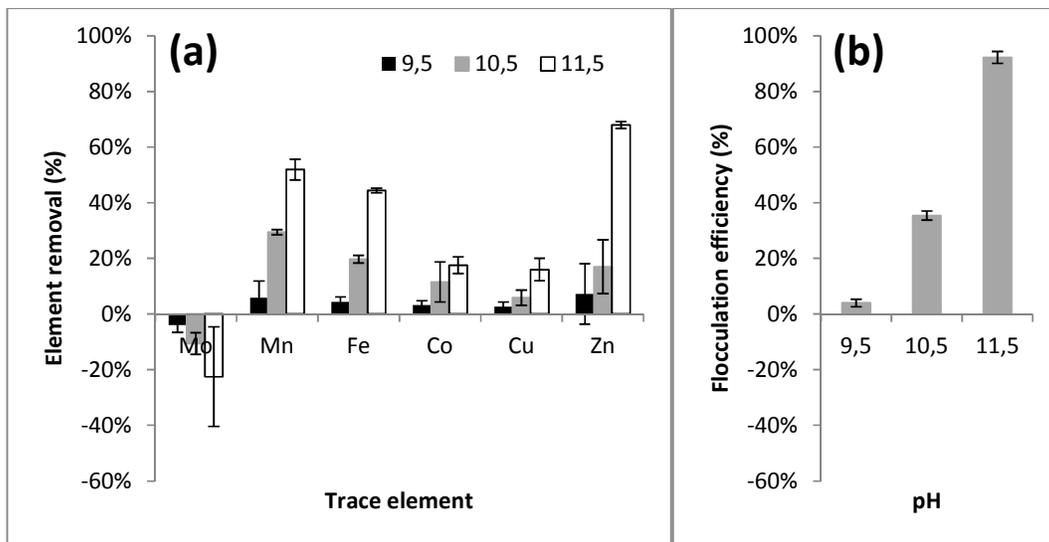
370 **Table 4. Influence of alkali species, phosphate concentration and pH on magnesium**  
 371 **and calcium removal during flocculation.**

Alkali	Phosphate concentration (μM)	pH	Mg removal (%)	Ca removal (%)
NaOH	2	9.5	-1.4 ± 0.02	44.5 ± 0.8
		10.5	3.55 ± 0.06	83.63 ± 0.0
		11.5	64.5 ± 0,1	90.4 ± 0.3
	350	9.5	-0.6 ± 0.1	23.4 ± 0.6
		10.5	5.7 ± 0.3	80.0 ± 0.2
		11.5	62.56 ± 0.06	88.4 ± 0.3
Ca(OH) <sub>2</sub>	2	9.5	0.9 ± 0.14	83.76 ± 0.07
		10.5	11.1 ± 0,1	95.3 ± 0.4
		11.5	67.3 ± 0.2	83.34 ± 0.02
	350	9.5	5.0 ± 0.5	87.97 ± 0.07
		10.5	14.2 ± 0.2	94.6 ± 0.1
		11.5	77.8 ± 0.4	82.70 ± 0.08

372

373 Trace elements removal

374 Removal of trace elements from the centrate due to NaOH flocculation was also  
 375 documented. Fig 7 shows that except for molybdenum, all trace elements were  
 376 removed in a manner dependent on pH level and correlated to flocculation efficiency  
 377 (ANOVA,  $P < 0.01$ ). Molybdenum is primarily present as the molybdate anion  $\text{MoO}_4^{2-}$ .  
 378 Unlike the other micronutrients, the dissolved molybdate concentration increases with  
 379 increasing pH (modelled with Visual MINTEQ, [33,34]).



380  
 381 **Fig 7. Trace element removal at different pH values (a) and comparison with**  
 382 **occurring flocculation efficiencies at corresponding pH (b) (n=2)**

383 *3.1.6. Practical implications of high pH induced flocculation*

384 Flocculation by addition of  $\text{Ca}(\text{OH})_2$  would be favoured to improve filterability of  
 385 centrate due to the higher flocculation ratio at intermediate pH values. At pH 10.5 at  
 386 low phosphate concentrations,  $\text{Ca}(\text{OH})_2$  flocculation was found to be 2.1 times more  
 387 effective compared to NaOH flocculation. In addition, lime is an economically  
 388 favourable and less hazardous alkali [38]. However, lime is more difficult to handle

389 because it is used as slurry which has to be constantly agitated. Furthermore,  
390 additional calcium is added to the centrate. Even after flocculation, slightly more  
391 calcium than the standard calcium concentration may remain dissolved, particularly if  
392 appreciable amounts of AOM are present in the centrate. Small excesses will  
393 accumulate with repeated medium recirculation, disturbing the broth composition.

394 NaOH flocculation is an alternative option. To obtain consistent high flocculation  
395 efficiencies, pH values up to 11.5 are needed. At this pH, flocculation is  $87 \pm 5\%$   
396 effective on average while sodium enrichment is moderate (maximum 14% of standard  
397 medium concentration). Furthermore, phosphate dependency is insignificant, thus,  
398 unsustainable phosphate addition is unnecessary. However, at high pH values,  
399 magnesium, calcium, phosphate and trace elements are quantitatively removed. By  
400 optimising production, residual phosphate can be minimized, reducing phosphate loss.  
401 An advantage of flocculation at high pH is the appreciable degree of disinfection  
402 occurring in the process. It was reported that at pH 11.0 – 11.5 and a contact time of  
403 4h, bacterial organisms are completely inactivated [39] and pathogenic  
404 microorganisms may be killed [40].

### 405 *3.2. Centrate clean-up by stand-alone sand filtration*

406 Only minor amounts of suspended solids were retained on a sand filter composed of  
407 35 cm of coarse sand (250 - 500  $\mu\text{m}$ ) topped with 5 cm of fine sand ( $D_{v50}$  of 170  $\mu\text{m}$ ).  
408 Over six separate runs, an average removal of  $7 \pm 4\%$  based on optical density was  
409 obtained. During filtration, the water height above the sand bed increased steadily  
410 with a final increase of  $22 \pm 4\%$  compared to the initial height. An average outlet flow

411 of  $8.2 \pm 0.6 \text{ m}^3 \cdot \text{m}^{-2} \cdot \text{h}^{-1}$  was maintained. Furthermore, the fouling index  $k_s$  (standard  
412 blocking) of collected permeate decreased with only  $3 \pm 2\%$  after 30 minutes and  $21 \pm$   
413  $21\%$  after 55 minutes. Removal rates of 90% up to 97.3% as reported by Sabiri et al.  
414 [22] and Naghavi et al. [23] for the sand filtration of microalgae were not obtained.  
415 Algae investigated in those studies had minimum diameters of 10 and 30  $\mu\text{m}$   
416 respectively. The results of this investigation indicate that some particles present in  
417 the centrate were retained on the sand filter but the overall removal is not sufficient  
418 to improve the filterability significantly.

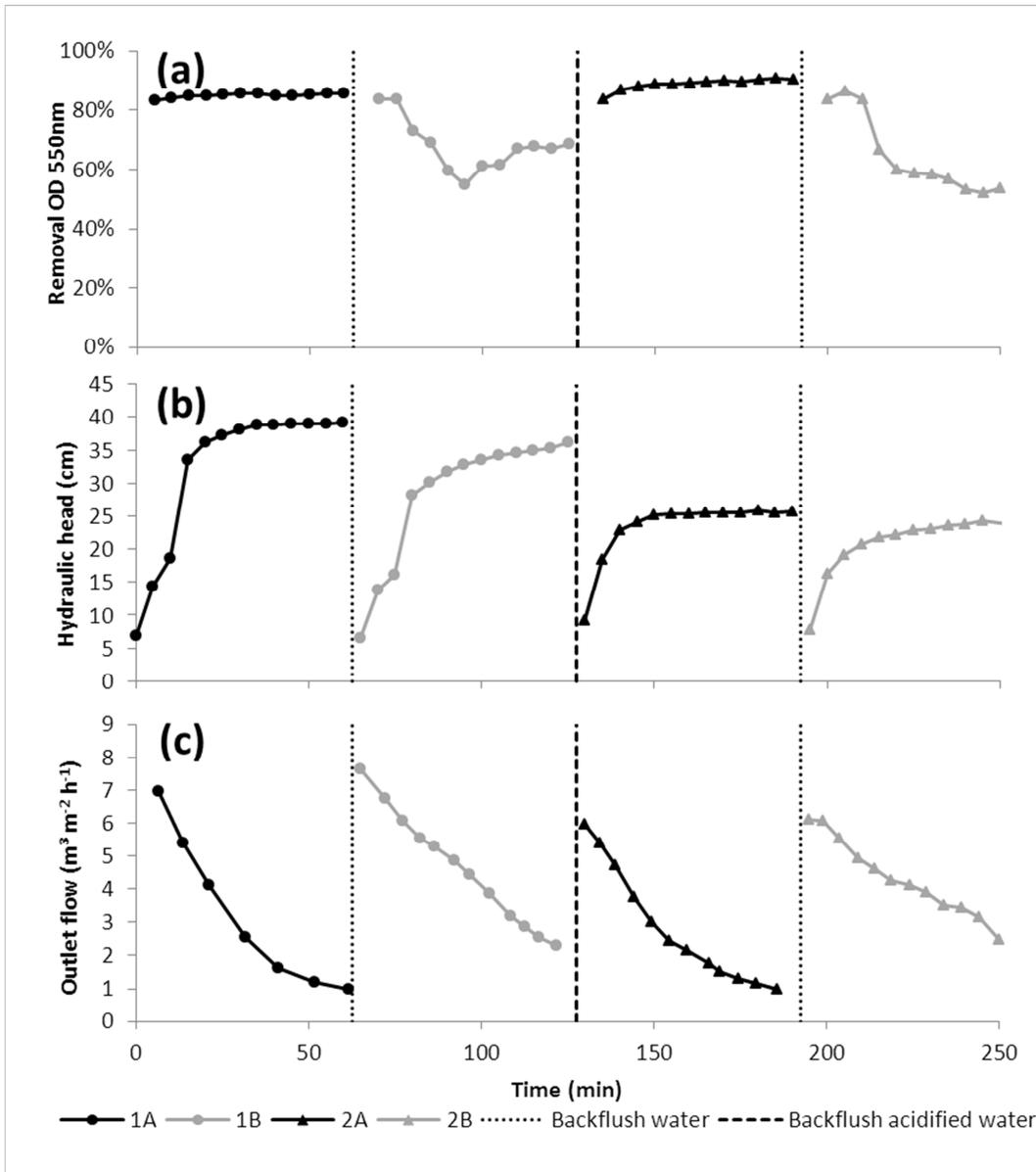
#### 419 *3.2.1. Nutrients retained by stand-alone sand filtration*

420 No nitrate and only very small amounts of phosphate were removed from the  
421 centrate,  $-0.2 \pm 0.9\%$  and  $2.3 \pm 1.9\%$  respectively. Likewise, no or only minute amounts  
422 of calcium and magnesium were retained on the sand,  $-1.1 \pm 0.1\%$  and  $0.81 \pm 0.03\%$   
423 respectively. Therefore, no extra cost would be generated to rejuvenate the centrate  
424 should one opt to solely use sand filtration for cleansing.

#### 425 *3.3. Centrate clean-up by combination of flocculation and sand filtration*

426 Sand filtration of centrate which was flocculated at pH of 10.5 or higher by addition of  
427  $\text{Ca}(\text{OH})_2$  resulted in rapid clogging of the sand bed, inhibiting filtration completely.  
428 Similar clogging was observed after flocculation at pH of 11.5 with NaOH.  
429 Consequently, only the combination of NaOH flocculation at pH 10.5 with sand  
430 filtration was further investigated. With a clean sand bed a reduction of  $87 \pm 2\%$  in  
431 optical density was obtained. However, removal rates of subsequent filtration runs

432 decreased rapidly until it was reduced to  $61 \pm 6\%$  (Fig 8a, run 1B and 2B). This  
433 decreased retention may have been caused by the build-up of clumps of sand that  
434 disturb the integrity of the sand bed. Retention capacity was completely restored after  
435 a cleaning of the sand bed with acidified water to disintegrate these clumps (Fig 8a,  
436 run 2A). Over all runs, on average,  $78 \pm 18\%$  of particles were removed and MFI's were  
437 improved with  $75 \pm 19\%$ . Under 'ideal' circumstances (A runs),  $87 \pm 2\%$  of particles  
438 were removed and MFI's were improved with  $84 \pm 14\%$ .



439

440 **Fig 8. Progression of particle removal (a), hydraulic head (b) and outlet flow (c) of**  
 441 **consecutive sand filtration runs of NaOH flocculated centrate at pH 10.5. The sand**  
 442 **bed was rinsed with acidified water between run 1B and 2A.**

443 During filtration, the centrate volume and pressure above the filtration surface

444 increased rapidly which indicates clogging of the filter (Fig 8b). The outlet flow

445 decreased accordingly (Fig 8c). This respective increase and decrease was less

446 pronounced in the secondary runs (1B, 2B), indicating a less clogged sand bed with

447 formation of preferential pathways. At the end of the runs, the filtration modulus had  
448 changed from rapid to slow sand filtration. The enhanced removal was partly due to  
449 mechanical straining of the aggregates in the sand bed. In addition, flocs accumulated  
450 on the surface of the sand forming an extra filtration layer, increasing the filtration  
451 efficiency of the sand filter.

452 *3.3.1. Practical implications of the combination of flocculation and sand*  
453 *filtration*

454 Our results in a lab-scale setup, are counter indicative for the combination of very high  
455 pH (>11) induced flocculation with sand filtration. A large scale sand filter can  
456 withstand higher pressure while providing a continuous inlet flow, which may sustain  
457 longer filtration runs with higher flows, but extensive test runs at pilot scale are  
458 needed to verify this.

459 Based on the results at hand, filtration through a sand bed consisting of 7 parts of  
460 coarse sand topped with 1 part of fine sand, preceded by NaOH induced flocculation at  
461 pH 10.5 is recommended. If under these conditions pressure still reaches the  
462 maximum operating pressure of 1.5 bar adopted in most sand filtration systems, the  
463 top layer can be removed. A small loss in filterability of the centrate will however  
464 occur. Flocculation with  $\text{Ca}(\text{OH})_2$  or at higher pH is best combined with a different  
465 separation technique or decantation.

466 The proposed method mainly removes particular matter from the medium. Small  
467 particles passing the sand filter are retained on the micro membrane. Dissolved  
468 organic material, however, is only partly removed from the medium. Preliminary

469 measurements showed that NaOH flocculation resulted in a removal of dissolved total  
470 organic carbon of  $25 \pm 3 \%$  at a pH of 10.5 and  $72 \pm 4 \%$  at a pH of 11.5. Remaining  
471 organics are recirculated back to the cultivation system. Hence, accumulation of these  
472 components may occur with extensive medium recycling. Their effect on productivity  
473 requires further investigation.

#### 474 *3.4. Cost calculation*

475 Based on the above, a combination of sand filtration and high pH induced flocculation  
476 is proposed as a means of pre-treatment for recycling used algae cultivation media.  
477 The additional cost of a sand filtration setup to an existing microfiltration setup, and  
478 consumption of chemicals and nutrients should therefore be compared to the  
479 replacement cost of fresh medium and the saved cost on filter membrane renewal.  
480 Table 5 gives an overview of the cost for a medium filtration setup applicable to a  
481 ProviAPT™ pilot production module of 0.288 ha. The installation cost for the  
482 membrane unit comprises three micro hollow fibre membranes and a circulation  
483 pump. The cost of the sand filtration unit contains a sand filter, sand, a circulation  
484 pump and a  $1\text{m}^3$  vessel to allow circulation of the centrate over the sand filter. To  
485 obtain the total installed cost, equipment costs were multiplied with a typical Lang  
486 factor for fluids processing of 4.74. Since the microfiltration unit is already part of the  
487 current setup to deliver contaminant-free fresh media, its installation and the  
488 associated costs do not add to the cost of its application for medium re-use. Actually,  
489 as a result of the suggested pre-treatments, the number of membrane module

490 replacements should become reduced. However, at present, data for an accurate  
491 impact estimate are not available.

492 Total flocculation cost was calculated by taking into account alkali cost to increase pH,  
493 cost of acid requirement to neutralise the medium and the replenishment cost of  
494 nutrients which were removed from the medium during the course of flocculation. By  
495 using NaOH instead of  $\text{Ca}(\text{OH})_2$  to increase the pH to 10.5 and 11.5 the chemical and  
496 nutrient cost is 6% and 21% cheaper respectively. The cost of chemicals and nutrient  
497 replenishment for NaOH induced flocculation at pH 10.5 and 11.5 is respectively  $1.12$   
498  $\text{€}\cdot\text{m}^{-3}$  and  $3.70 \text{€}\cdot\text{m}^{-3}$  of treated centrate. Sand filtration adds approximately  $0.18 \text{€}\cdot\text{m}^{-3}$ .  
499 These costs are relatively small compared to the current cost of fresh medium of  $13.21$   
500  $\text{€}\cdot\text{m}^{-3}$ . It should be noted that fresh medium is composed of expensive artificial food  
501 grade salts ( $9.32 \text{€}\cdot\text{m}^{-3}$  medium), food grade nutrients ( $2.12 \text{€}\cdot\text{m}^{-3}$  medium) and water  
502 ( $1.77 \text{€}\cdot\text{m}^{-3}$  medium). Assuming a recycling rate of 75%, the medium cost decreases to  
503  $4.32 \text{€}\cdot\text{m}^{-3}$ . Complete rejuvenation of all nutrients is included in this cost.

504 For a 0.288 ha production installation of the ProviAPT™ system, flocculation at a pH of  
505 10.5 and subsequent sand filtration make for an additional running cost of  $0.44 \text{€}\cdot\text{kg}^{-1}$   
506 DW. Medium costs constitute  $1.62 \text{€}\cdot\text{kg}^{-1}$  DW or  $0.95 \text{€}\cdot\text{kg}^{-1}$  DW when respectively 75%  
507 or 90% of medium is reused. At 75% recycle the total cost the proposed scheme of  
508  $2.08 \text{€}\cdot\text{kg}^{-1}$  DW would mean a 58% cut in cost compared to the total fresh medium cost  
509 of  $4.95 \text{€}\cdot\text{kg}^{-1}$  DW. With a cost of  $1.39 \text{€}\cdot\text{kg}^{-1}$  DW at 90% recycle, a gain of 72% can be  
510 accomplished.

511 **Table 5: Summarized cost for installation of filtration equipment, chemical usage for**  
 512 **flocculation, nutrient addition after flocculation and medium cost**

**Installation cost**

Unit	Cost (euro)
Membrane filtration unit (Lang factor 4.74)	67826
Micro membrane filter: 11.2m <sup>2</sup> 0.2μm (n=3)	13468
Circulation pump	841
Sand filtration unit (Lang factor 4.74)	8107
Sand filter	795
Sand	108
Circulation pump	355
1m <sup>3</sup> buffer vessel	452

**Energy cost**

Unit	Cost (euro·m <sup>-3</sup> )
Membrane filtration unit: circulation pump	0.025
Sandfiltration unit: circulation pump	0.01

**Chemical usage flocculation**

Flocculation pH	Chemical	Consumption (kg·m <sup>-3</sup> )	Cost (euro·m <sup>-3</sup> )
11.5	NaOH	2.3	0.87
	Ca(OH) <sub>2</sub>	3.4	1.02
	HCl	0.41	0.44
10.5	NaOH	0.8	0.30
	Ca(OH) <sub>2</sub>	1.3	0.39
	HCl	0.15	0.16

**Extra nutrient usage**

Alkali and flocculation pH	Salt	Consumption (kg·m <sup>-3</sup> )	Cost (euro·m <sup>-3</sup> )
NaOH (11.5)	MgCl <sub>2</sub> ·6H <sub>2</sub> O	2.70	0.76
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.28	1.44
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.47	0.19
Ca(OH) <sub>2</sub> (11.5)	MgCl <sub>2</sub> ·6H <sub>2</sub> O	3.03	0.85
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.67	1.61
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0	0
NaOH (10.5)	MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.59	0.17
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.71	0.31
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.44	0.18

Ca(OH) <sub>2</sub> (10.5)	MgCl <sub>2</sub> ·6H <sub>2</sub> O 0.87	0.24
	MgSO <sub>4</sub> ·7H <sub>2</sub> O 1.06	0.47
	CaCl <sub>2</sub> ·2H <sub>2</sub> O 0.37	0.15
<b>Medium cost</b>		
Medium		Cost (euro·m <sup>-3</sup> )
100% fresh		13.21
75% recycle		4.32
90% recycle		2.54

513

#### 514 **4. Conclusion**

515 The main goal of this study consisted in finding a polishing treatment of centrate  
516 originating from harvest operations of high density cultures of *Nannochloropsis* sp. to  
517 feed it into a microfiltration setup generating contaminant-free growth medium. A  
518 combination of NaOH flocculation at pH 10.5 and subsequent sand filtration removed  
519  $78 \pm 18\%$  of particles and improved ‘modified fouling indices’ with  $75 \pm 19\%$ . Our  
520 results indicate that there is room for some improvement by optimization of washing  
521 regimes. In any case, the treatment will greatly decrease fouling and deterioration of  
522 filter membrane, thus allowing prolonged recirculation of growth medium. During such  
523 prolonged medium recycling, dissolved organic components may accumulate in the  
524 medium, the effect of which on productivity requires further investigation. Yet, at a  
525 sustained recycle rate of 90%, a cost saving of  $3.56 \text{ €} \cdot \text{kg}^{-1} \text{ DW}$ , i.e. 72% of the fresh  
526 medium cost, can be achieved.

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