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Validation of a cationic polyacrylamide flocculant for the harvesting fresh and seawater microalgal biomass

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27 **Abstract**

28 A simple, efficient, and fast settling flocculation technique to harvest microalgal biomass was
29 demonstrated using a proprietary cationic polyacrylamide flocculant for a freshwater (*Chlorella*
30 *vulgaris*) and a marine (*Phaeodactylum tricornutum*) microalgal culture at their mid-stationary
31 growth phase. The optimal flocculant doses were 18.9 and 13.7 mg/g of dry algal biomass for
32 *C. vulgaris* and *P. tricornutum*, respectively (equivalent to 7 g per m^3 of algal culture for both
33 species). The obtained optimal dose was well corroborated with changes in cell surface charge,
34 and culture solution optical density and turbidity. At the optimal dose, charge neutralization of
35 64 and 86% was observed for *C. vulgaris* and *P. tricornutum* algal cells, respectively. Algae
36 recovery was independent of the culture solution pH in the range of pH 6 to 9. Algal biomass
37 recovery was achieved of 100 and 90% for *C vulgaris* and *P. tricornutum* respectively, and over
38 98% medium recovery was achievable by simple decanting.

39

40 **Keywords**: *Chlorella vulgaris*; *Phaeodactylum tricornutum*; Cationic polyacrylamide
41 polymer; Biomass recovery; Algae harvesting.

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54 ## 1. Introduction

55 Microalgal biomass is a renewable feedstock for the production of biochemicals for food
56 additives and the biotechnology industry, animal feed, and biofuel (Vo et al., 2018; Vadivel et
57 al., 2019; Jacob-Lopes et al., 2019; Poddar et al., 2018; Khalid et al., 2019; Ma et al., 2018).
58 Microalgae production includes two major steps, namely culturing and harvesting. As progress
59 has been achieved to optimize growth condition and nutrient requirements for effective
60 microalgae culturing, the second step has emerged as a major bottleneck for cost-effective
61 microalgal biomass production. Large-scale harvesting of microalgal biomass is challenging
62 due to low cell concentrations (less than 1 g/L in a mature culture), small cell sizes (3–30 µm),
63 stability of cell suspension, and complex culturing solution matrix (Vandamme et al., 2010;
64 Zheng et al., 2012; Li et al., 2017a; Augustine et al., 2019; Muylaert et al., 2017). An estimation
65 of 30% of the total production cost is attributed to microalgal harvesting (Şirin et al., 2012),
66 which is arguably the most energy-intensive step in the production of microalgal-based
67 materials.

68 Several microalgae harvesting techniques including membrane filtration, centrifugation and
69 flocculation have been explored and reported in the literature (Vandamme et al., 2010; Şirin et
70 al., 2012; Rashid et al., 2013; Bilad et al., 2012). Amongst them, flocculation is the most
71 promising option for low cost microalgae harvesting, although the biomass recovery efficiency
72 is often low (Vandamme et al., 2010; Şirin et al., 2012; Rashid et al., 2013; Pandey et al., 2019;
73 Ummalyma et al., 2017). Common flocculants for microalgae harvesting can be, divided into
74 three groups: i) inorganics such as ferric chloride and aluminium sulfate; ii) synthetic polymers
75 such as polyacrylamide and polyethyleneimine; and iii) bio-agent flocculants such as fungi,
76 protein, and chitosan (Vandamme et al., 2010; Rashid et al., 2013; Horiuchi et al., 2003; Li et
77 al., 2017b). Inorganic flocculants are required at high doses (up to g/L) and increase the
78 impurity of microalgal biomass, limiting its application and necessitating downstream
79 processing (Şirin et al., 2012). Recently, the second and third groups of flocculants have been

80 extensively studied. Performances of these flocculants are often dependent on pH, long settling
81 time and growth medium matrix (freshwater *vs* seawater). Vandanme et al. (2010) reported that
82 cationic starch could effectively recover freshwater *Parachlorella* and *Scenedesmu* but not
83 marine microalgae such as *Phaedactylum* and *Nannochloropsis*. Likewise, chitosan can be
84 effective for harvesting marine microalgae at high dose (e.g. 40 mg/L or more) (Cheng et al.,
85 2011) . The culture media matrix (i.e. ionic strength, cell structure of fresh and marine
86 microalgae) influences the efficiency of previous flocculants (Pandey et al., 2019; Roselet et
87 al., 2015). Moreover, previous studies reported that a relatively higher dose of inorganic and
88 polymer flocculant is needed for marine microalgae (Bilanovic et al., 1988; Fabrizi et al., 2010;
89 Uduman et al., 2010). Given the performance of current available flocculants and the wide
90 range of applications of marine microalgal-based products, it is essential to identify a versatile
91 flocculant that can be used for both freshwater and marine microalgae, over a wide pH range
92 and at low doses.

93 This study aims to validate the efficiency of a cationic polymer on the recovery of the
94 freshwater (*Chlorella vulgaris*) and marine (*Phaeodactylum tricornutum*) microalgae. The
95 polymer has been widely used in the water industry but has not been applied to harvest
96 microalgae. A dose-response experiment was performed to determine the optimal polymer
97 dose. Optical density removal, turbidity, zeta potential and biomass recovery were examined to
98 evaluate flocculation efficiency and mechanisms.

99 **2. Materials and methods**

100 2.1 Microalgae strains and growth conditions

101 The freshwater green algae *C. vulgaris* (CS-41) was obtained from
102 the Australian National Algae Culture Collection, CSIRO Microalgae Research (Hobart, TAS,
103 Australia) and marine diatom *P. tricornutum* (CCMP 632) was obtained from the National
104 Centre for Marine Algae and Microbiota (NCMA) (East Boothbay, ME, USA). They were
105 maintained at the Climate Change Cluster (C3) culture collection at University Technology

106 Sydney in freshwater MLA media and f/2 (marine) media (Algaboost; Wallaroo, SA, Australia)
107 using seawater collected from Sydney Harbour (salinity of 33-35 g/L) respectively. Seed
108 cultures were grown up to early stationary phase in 1-L Schott's bottles, followed by 10-L
109 carboys, bubbled with air in ~20 °C and ~100 µmol/m^2/s light in a 16:8 hour light:dark cycle.
110 *P. tricornutum* was harvested from the carboy, while *C. vulgaris* was further scaled up with a
111 1/50 inoculation in a 400-L bag bioreactor using dechlorinated tap water with Jaworski medium
112 (Fresh by Design; Moss Vale, NSW, Australia), in a temperature controlled room of ~20 °C
113 and ~400 µmol/m^2/s light in a 16:8 hour light:dark cycle. This seeding protocol was adapted to
114 sequentially scale up the reactor volume, maintaining sufficient initial microalgal biomass
115 (Pereira et al., 2018). For media under 10 L, the media were sterilized before inoculation by
116 filtering the seawater through a Whatman 0.2 µm filter, and both fresh and marine water was
117 then autoclaved, followed by addition of filter sterilized stock media components through a
118 Whatman 0.2 µm filter. For the large scale bag, the water was sterilized with 100 mL 12%
119 sodium hypochlorite, followed by 100 mL 2 M sodium thiosulphate. The algae were checked
120 for pH twice a day, and if the pH reached above 9.4, the culture was sparged with CO_2 up to 3
121 minutes. The rate of microalgae growth was monitored every day by measuring optical density
122 at wavelengths of 680 and 730 nm for *C. vulgaris* and *P. tricornutum*, respectively (Nguyen &
123 Rittmann, 2018). The harvesting experiments were performed with the microalgae culture at a
124 mid-stationary phase.

125 2.2 Experimental set up for flocculation

126 A cationic polyacrylamide flocculant (FO3801) with high-charge (>80% charge) high-
127 molecular weight (>15 Megadalton) (SNF Pty Ltd; Corio, VIC, Australia) was used in this
128 study. A stock solution of the polymer (0.4% v/v) was prepared in Milli-Q water with
129 continuous mixing at 100 rpm for 1 h and stored at room temperature and used within 1 day of
130 preparation.

131 Microalgae cell suspensions at a mid-stationary growth phase (Section 2.1) were used for all
132 harvesting experiments. Different polymer doses of 2.7 to 54 mg polymer/g biomass (dry
133 weight) (i.e. 1 to 20 mg/L) were added in glass bottles containing *C. vulgaris*. Likewise,
134 polymer doses of 1.97 to 39.4 mg polymer/g biomass (dry weight) (i.e. 1 to 20 mg/L) was used
135 for *P. tricornutum*. The bottles were then gently mixed by hand for one minute and then allowed
136 to settle for another minute. An aliquot (10 mL) of the culture in the bottle was pipetted from a
137 height of one- and two-thirds from the bottom for evaluating the flocculation performance.
138 The flocculation efficiency was calculated based on the change in the optical density at
139 wavelength of 680 and 730 nm before and after each polymer addition, as shown in the
140 following equation.

141 $$\text{Flocculation efficiency (\%)} = \left(\frac{OD_i - OD_f}{OD_i}\right) \times 100$$

142 Where OD_i and OD_f is the optical density of the culture before and after flocculant addition.
143 Each polymer dose was repeated three times for two different microalgae cultures.
144

145 ## 2.3 Analytical methods

146 The optical density of microalgae medium before and after was measured by the UV
147 spectrophotometer (UV 6000 Shimadzu; Ermington, NSW, Australia). The wavelengths were
148 680 and 730 nm for *C. vulgaris* and *P. tricornutum*, respectively.
149 The biomass concentration was determinded gravimetrically by drying the sample to a
150 constant mass at 60 °C within 4 h. A 150 mL aliquot of microalgae cells suspension was filtered
151 through a 1.1 μm pre-weighed glass fiber filter paper. The weight of the final filter paper was
152 used to calculate the dry microalgal biomass. This protocol was also applied for the
153 determination of biomass volume after flocculation. For marine *P. tricornutum* culture, Milli-
154 Q water (100 mL) was used to rinse (i.e. salt removal) before drying.

155 Zeta potential of the microalgae solutions before and after flocculation was measured using
156 a Zetasizer nano instrument (Nano ZS Zen 3600; Malvern, UK). The zeta potential values were
157 measured using a 10 mL sample of cells suspensions (i.e. initial sample) and 10 mL of
158 supernatant after flocculation. All samples were at pH 9.5.

159 The solution pH was measured using a pH/conductivity meter (Orion 4-Star Plus Thermo
160 Scientific; Waltham, MA, USA). The pH adjustment was achieved by using 0.1 M NaOH and
161 0.1 M H_2SO_4. Turbidity of the microalgae solution before and after flocculation was measured
162 using a portable turbidity meter kit (Apera TN400; Colombus, OH, USA) with accuracy ±1%
163 or 0.02 NTU.

164 Statistical analysis was performed in Microsoft Excel using Student's unpaired *t*-Test, with
165 a two-tailed distribution.

166 **3. Results and discussion**

167 3.1 Polymer dose optimisation

168 A dose-response relationship revealed the optimal flocculation efficiency at polymer dose
169 of 18.9 and 13.7 mg/g dry biomass for *C. vulgaris* and *P. tricornutum* (i.e. equivalent to 7 mg/L
170 for both freshwater *C. vulgaris* and marine *P. tricornutum* (Fig. 1)). The optical density OD_{680}
171 removal increased gradually from 44 to 90% with polymer dose of 2.7 and 18.9 mg/g in the *C.*
172 *vulgaris* solution. A further increase in polymer dose up to 54 mg/g did not result in the
173 improvement of optical density removal (Fig. 1). Similarly, the optical density removal
174 increased from 82 to 99% with polymer dose of 1.9 to 13.7 mg/g in the *P. tricornutum* solution.
175 At 39.4 mg/g, the OD_{730} removal was 75%, which is statistically significantly lower than that
176 at 13.7 mg/g (unpaired *t*-test, p-value < 0.05). The data in Fig. 1 suggest that polymer over-
177 dosing can be counterproductive.

178 [FIGURE 1]

179 Results from Fig. 1 demonstrate that the proprietary high charge, high molecular weight
180 cationic polyacrylamide can be used for both fresh and marine microalgae flocculation. The

181 optimal doses were 18.9 and 13.7 mg/g dry biomass for *C. vulgaris* and *P. tricornutum*,
182 respectively. The mid stationary biomass were at 370 mg/L for *C. vulgaris*, and 508 mg/L for
183 *P. tricornutum*. As such, the optimal dose is equivalent to 7 mg/L of culture solution for both
184 species. Several previous studies have established 90% optical density removal as the
185 benchmarking value for effective flocculation (Ma et al., 2018; Roselet et al., 2015; Uduman et
186 al., 2010). In this study, 90 to 99% optical density removal was achievable for both freshwater
187 and marine microalgae.

188 Although the optimal dose varies from flocculant to flocculant, a considerably lower optimal
189 dose was obtained in this study compared to the literature. For example, the optimal dose of an
190 organic chitosan flocculant reported by several previous studies (Augustine et al., 2019; Rashid
191 et al., 2013) was 120 mg/L (equivalent to approximately 120 mg/g dry algae biomass), which
192 is 6.3 times higher than the optimal dose for the *C. vulgaris* in this study. Flocculation efficiency
193 of chitosan for the marine microalgae *P. tricornutum* was below 30% at a dose of 30 mg/L (Ma
194 et al., 2018; Şirin et al., 2012).

195 Another indicator of the efficiency of cationic polyacrylamide is the settling time. In this
196 study, effective flocculation was observed within 1 min of polymer dosing. This appears to be
197 the fastest settling time in the literature (Augustine et al., 2019; Rashid et al., 2013; Pandey et
198 al., 2019). Pandey et al. (2019) observed maximum flocculation after 40 to 60 min settling time
199 using Al^{3+}, Ca^{2+} and egg cell powder. The fast settling time achieved with the proprietary
200 polymer in our study provides an opportunity to integrate the harvesting process into the
201 microalgal culturing in a continuous system that likely enhances the commercialization of
202 microalgal industry. In addition, Xiong et al. (2018) suggested that the polyacrylamide
203 backbone of this proprietary polymer is non-toxic and can readily be hydrolyzed once dissolved
204 in water. This property can potentially expand the usage of cationic polyacrylamide on stringent
205 applications of the harvested microalgae (e.g. food additives and cosmetic reagents).
206 Nevertheless, further study is necessary to assert these applications.

207 3.2 Flocculation mechanisms

208 Microalgae cell neutralisation is the main mechanism for the flocculation formation with
209 cationic polyacrylamide (Fig. 2). The charges of growth media increased gradually with
210 polymer levels. The *C. vulgaris* and *P. tricornutum* microalgae cells are negatively charged at
211 -15.6 and -12.6 mV, respectively. The negative surface charge of microalgae cells in a culture
212 suspension is induced by the carboxylic and sulfate functional groups on the microalgae surface
213 (Ndikubwimana et al., 2015). When they are highly negatively charged, electrostatic repulsion
214 maintains suspension amongst cells. When a cationic polymer is added, the electrostatic
215 repulsion decreased (as indicated by zeta potential) (Fig 2), promoting flocculation. The zeta
216 potential data corroborated with the optimal polymer dose (Section 3.1). At the optimal doses
217 18.9 and 13.7 mg/g, zeta potentials changed from -15.6 to -5.6 (i.e. 64% change) and from -
218 12.6 to -1.7 (i.e. 86% change) in *C. vulgaris* and *P. tricornutum*, respectively. Charge
219 neutralization of the microalgal suspension has been observed as a major flocculation
220 mechanism in previous studies using polymeric flocculants. For example, Zheng et al. (2012)
221 observed an increase in zeta potential from -19 to 0.8 after addition of 20 mg/L y-glutamic acid
222 to a *C. vulgaris* suspension.

223 [FIGURE 2]

224 Results from Fig. 2 suggest that complete neutralization of microalgae cells suspension is
225 not necessary for effective flocculation. The microalgal suspension reached an isoelectric point
226 at polymer doses of 45.9 and 16.2 mg/g dry biomass for *C. vulgaris* and *P. tricornutum*,
227 respectively. These doses were 2.4 and 1.2 times higher than the optimal dose for *C. vulgaris*
228 and *P. tricornutum*, respectively. Over dosing of a polymer can cause charge reversal of the
229 microalgae cells and thus decrease the flocculation efficiency. This process, called
230 restabilization, has also been observed with other polymeric flocculants such as cationic starch
231 (Vandamme et al., 2010) and chitosan (Rashid et al., 2013). The restabilization did not occur

232 in this study at a concentration range of 7 to 20 mg/L (i.e. even after the isoelectric point),
233 suggesting that the cationic polyacrylamide can be used over a wide range of doses.
234 The microalgal culture medium can influence neutralization and thus, flocculation
235 efficiency. The high ionic strength of seawater (i.e. marine microalgal culture) reduced the
236 flocculation efficiency of cationic polymers (Roselet et al., 2015; Bilanovic et al., 1988; König
237 et al., 2014). König et al. (2014) reported that medium salinity reduction (50%) could improve
238 the flocculation of polymeric Zetag® and marine microalgal *Chlorella stigmatophora*.
239 However, the polymer used in this study can perform effectively well with both freshwater and
240 marine species, thus can be potentially applied to other microalgae.

241 3.3 Impact of pH on flocculation efficiency

242 The flocculation efficiency of cationic polyacrylamide with *C. vulgaris* and *P. tricornutum*
243 was independent of pH ranging from 6 to 9 (Table 1). No statistically significant difference
244 (unpaired *t*-test, p-value > 0.05) in optical density removal, NTU and zeta potential was
245 observed at pH 6 to 9. The pH of microalgal culture can vary depending on different growth
246 phase, an alkalinity level of growth medium and the concentration of dissolved carbon dioxide.
247 The obtained results show that no pH adjustment would be required for the microalgal
248 harvesting in this study within a pH range of 6-9.

249 [TABLE 1]

250 Changing pH values of the microalgae growth medium is often required for effective
251 flocculation as shown in previous studies (Horiuchi et al., 2003; Ummalyma et al., 2016; Lal &
252 Das, 2016; Tran et al., 2017). For example, the cell surface charge of *Chlorella* species
253 decreases at low pH (Lal & Das, 2016). At a pH of 5.5, the amine groups in extracellular
254 polysaccharides, protein and lipids dissociate, and the carboxylic group is used to protect the
255 dissociation. This process decreases the negative charge of microalgal cells (Tran et al., 2017).
256 Increasing the pH values can induce chemical precipitation of calcium and magnesium salts in
257 the culture medium. Chemical precipitation can increase the impurity of the final microalgal

258 products. Adjusting pH during the harvesting process also change the cell integrity and the
259 downstream process (Augustine et al., 2019; Şirin et al., 2012). A flocculant that can work
260 effectively without pH adjustment is desirable. The finding of this study, therefore, suggests
261 such flocculant exists and, to our knowledge, this is the first time it is reported.

262 3.4 Final biomass harvesting

263 The microalgal biomass recoveries were 100 and 90 ± 2.3% for *C. vulgaris* and *P.*
264 *tricornutum*, respectively (Fig. 3a). At lab-scale testing, the addition of polymer at 7 mg/L (i.e.
265 at a cost of 0.049 $AUD per m^3) into both freshwater and marine microalgal cultures formed
266 very stable flocs (i.e. without any alterations after 24 h). The solutions can be easily decanted
267 to remove water (Fig 3b&c). Simultaneously, above 98% recovery of the culture medium was
268 achieved in this study. The results from this study provide conclusive evidence that cationic
269 polyacrylamide that has been widely used for sludge dewatering can be also used for microalgal
270 biomass and culture medium separation.

271 [FIGURE 3]

272

273 **4. Conclusions**

274 This study demonstrates the effectiveness of a proprietary high charge high molecular weight
275 cationic polyacrylamide for simple, robust, and efficient recovery of freshwater and marine
276 microalgae *C. vulgaris* and *P. tricornutum*. A dose-response relationship showed that the
277 optimal polymer doses were 18.9 and 13.7 mg/g dry biomass. At the optimal dose, microalgal
278 cell surface charge was neutralised at 64 and 86% for *C. vulgaris* and *P. tricornutum,*
279 respectively. Between pH 6 and 9, the solution pH did not affect flocculation efficiency. Thus,
280 this method can be used for both freshwater and marine microalgae species over a wide range
281 of culture conditions.

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286 **6. Declarations**

287 The authors declare that there is no conflict of interest regarding the publication of this

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289 **References**

290 [1] Vo, P., Ngo, H.H., Guo, W.S., Chang, S.W., Nguyen, D.D., Nguyen, P.D., Bui, X.T., Zhang, X.B.,
291 Guo, J.B. 2018. Can algae-based technologies be an affordable green process for biofuel
292 production and wastewater remediation? Bioresour. Technol., 256, 491-501.
293 [2] Vadivel, M., Arvindnarayan, S., Kumar, G., Shobana, S., Dharmaraja, J., Nguyen, D.D., Chang,
294 S.W., Prabhu, K.K.S. 2019. Biodiesel Potentiality of Microalgae Species: evaluation Using
295 Various Nitrogen Sources. Waste and Biomass Valorization.
296 [3] Jacob-Lopes, E., Maroneze, M.M., Deprá, M.C., Sartori, R.B., Dias, R.R., Zepka, L.Q. 2019.
297 Bioactive food compounds from microalgae: an innovative framework on industrial
298 biorefineries. Current Opinion in Food Science, 25, 1-7.
299 [4] Poddar, M.K., Anand, M., Farooqui, S.A., Martin, G.J.O., Maurya, M.R., Sinha, A.K. 2018.
300 Hydroprocessing of lipids extracted from marine microalgae Nannochloropsis sp. over
301 sulfided CoMoP/Al2O3 catalyst. Biomass Bioenergy, 119, 31-36.
302 [5] Khalid, A.A.H., Yaakob, Z., Abdullah, S.R.S., Takriff, M.S. 2019. Assessing the feasibility of
303 microalgae cultivation in agricultural wastewater: The nutrient characteristics. Environmental
304 Technology & Innovation, 15, 100402.
305 [6] Ma, Y., Gao, Z., Wang, Q., Liu, Y. 2018. Biodiesels from microbial oils: Opportunity and
306 challenges. Bioresour. Technol., 263, 631-641.
307 [7] Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K. 2010. Flocculation of microalgae using
308 cationic starch. Journal of Applied Phycology, 22(4), 525-530.
309 [8] Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X., Huang, H. 2012. Harvesting of microalgae by
310 flocculation with poly (γ-glutamic acid). Bioresour. Technol., 112, 212-220.
311 [9] Li, Y., Xu, Y., Liu, L., Li, P., Yan, Y., Chen, T., Zheng, T., Wang, H. 2017a. Flocculation
312 mechanism of Aspergillus niger on harvesting of Chlorella vulgaris biomass. Algal Research,
313 25, 402-412.

314 [10] Augustine, A., Tanwar, A., Tremblay, R., Kumar, S. 2019. Flocculation processes optimization
315 for reuse of culture medium without pH neutralization. Algal Research, 39, 101437.
316 [11] Muylaert, K., Bastiaens, L., Vandamme, D., Gouveia, L. 2017. 5 - Harvesting of microalgae:
317 Overview of process options and their strengths and drawbacks. in: *Microalgae-Based*
318 *Biofuels and Bioproducts*, (Eds.) C. Gonzalez-Fernandez, R. Muñoz, Woodhead Publishing,
319 pp. 113-132.
320 [12] Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J. 2012. Harvesting the microalgae Phaeodactylum
321 tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced
322 flocculation. Journal of Applied Phycology, 24(5), 1067-1080.
323 [13] Rashid, N., Rehman, S.U., Han, J.-I. 2013. Rapid harvesting of freshwater microalgae using
324 chitosan. Process Biochem., 48(7), 1107-1110.
325 [14] Bilad, M.R., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I.F.J. 2012. Harvesting
326 microalgal biomass using submerged microfiltration membranes. Bioresour. Technol., 111,
327 343-352.
328 [15] Pandey, A., Pathak, V.V., Kothari, R., Black, P.N., Tyagi, V.V. 2019. Experimental studies on
329 zeta potential of flocculants for harvesting of algae. J. Environ. Manage., 231, 562-569.
330 [16] Ummalyma, S.B., Gnansounou, E., Sukumaran, R.K., Sindhu, R., Pandey, A., Sahoo, D. 2017.
331 Bioflocculation: An alternative strategy for harvesting of microalgae – An overview.
332 Bioresour. Technol., 242, 227-235.
333 [17] Horiuchi, J.-I., Ohba, I., Tada, K., Kobayashi, M., Kanno, T., Kishimoto, M. 2003. Effective cell
334 harvesting of the halotolerant microalga Dunaliella tertiolecta with pH control. J. Biosci.
335 Bioeng., 95(4), 412-415.
336 [18] Li, Y., Xu, Y., Zheng, T., Wang, H. 2017b. Flocculation mechanism of the actinomycete
337 Streptomyces sp. hsn06 on Chlorella vulgaris. Bioresour. Technol., 239, 137-143.
338 [19] Cheng, Y.-S., Zheng, Y., Labavitch, J.M., VanderGheynst, J.S. 2011. The impact of cell wall
339 carbohydrate composition on the chitosan flocculation of Chlorella. Process Biochem., 46(10),
340 1927-1933.
341 [20] Roselet, F., Vandamme, D., Roselet, M., Muylaert, K., Abreu, P.C. 2015. Screening of
342 commercial natural and synthetic cationic polymers for flocculation of freshwater and marine
343 microalgae and effects of molecular weight and charge density. Algal Research, 10, 183-188.
344 [21] Bilanovic, D., Shelef, G., Sukenik, A. 1988. Flocculation of microalgae with cationic polymers
345 — Effects of medium salinity. Biomass, 17(1), 65-76.
346 [22] Fabrizi, L., Jefferson, B., Parsons, S.A., Wetherill, A., Jarvis, P. 2010. The Role of Polymer in
347 Improving Floc Strength for Filtration. Environ. Sci. Technol., 44(16), 6443-6449.
348 [23] Uduman, N., Qi, Y., Danquah, M.K., Hoadley, A.F.A. 2010. Marine microalgae flocculation and
349 focused beam reflectance measurement. Chem. Eng. J., 162(3), 935-940.
350 [24] Pereira, H., Páramo, J., Silva, J., Marques, A., Barros, A., Maurício, D., Santos, T., Schulze, P.,
351 Barros, R., Gouveia, L., Barreira, L., Varela, J. 2018. Scale-up and large-scale production of

352 Tetraselmis sp. CTP4 (Chlorophyta) for CO2 mitigation: from an agar plate to 100-m3
353 industrial photobioreactors. Sci. Rep., 8(1), 5112.
354 [25] Nguyen, B.T., Rittmann, B.E. 2018. Low-cost optical sensor to automatically monitor and control
355 biomass concentration in microalgal cultivation. Algal Research, 32, 101-106.
356 [26] Xiong, B., Loss, R.D., Shields, D., Pawlik, T., Hochreiter, R., Zydney, A.L., Kumar, M. 2018.
357 Polyacrylamide degradation and its implications in environmental systems. npj Clean Water,
358 1(1), 17.
359 [27] Ndikubwimana, T., Zeng, X., He, N., Xiao, Z., Xie, Y., Chang, J.-S., Lin, L., Lu, Y. 2015.
360 Microalgae biomass harvesting by bioflocculation-interpretation by classical DLVO theory.
361 Biochem. Eng. J., 101, 160-167.
362 [28] König, R.B., Sales, R., Roselet, F., Abreu, P.C. 2014. Harvesting of the marine microalga
363 Conticribra weissflogii (Bacillariophyceae) by cationic polymeric flocculants. Biomass
364 Bioenergy, 68, 1-6.
365 [29] Ummalyma, S.B., Mathew, A.K., Pandey, A., Sukumaran, R.K. 2016. Harvesting of microalgal
366 biomass: Efficient method for flocculation through pH modulation. Bioresour. Technol., 213,
367 216-221.
368 [30] Lal, A., Das, D. 2016. Biomass production and identification of suitable harvesting technique for
369 Chlorella sp. MJ 11/11 and Synechocystis PCC 6803. 3 Biotech, 6(1), 41-41.
370 [31] Tran, N.-A.T., Seymour, J.R., Siboni, N., Evenhuis, C.R., Tamburic, B. 2017. Photosynthetic
371 carbon uptake induces autoflocculation of the marine microalga Nannochloropsis oculata.
372 Algal Research, 26, 302-311.

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