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Alkaline flocculation of *Phaeodactylum tricornutum* induced by brucite and calcite

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1 **Alkaline flocculation of *Phaeodactylum tricornutum* induced by brucite and**  
2 **calcite**

3

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17

18 **Abstract**

19

20 Alkaline flocculation holds great potential as a low-cost harvesting method for marine  
21 microalgae biomass production. Alkaline flocculation is induced by an increase in pH  
22 and is related to precipitation of calcium and magnesium salts. In this study, we used  
23 the diatom *Phaeodactylum tricornutum* as model organism to study alkaline  
24 flocculation of marine microalgae cultured in seawater medium. Flocculation started  
25 when pH was increased to 10 and flocculation efficiency reached 90% when pH was  
26 10.5, which was consistent with precipitation modeling for brucite or  $Mg(OH)_2$ .  
27 Compared to freshwater species, more magnesium is needed to achieve flocculation  
28 ( $> 7.5$  mM). Zeta potential measurements suggest that brucite precipitation caused  
29 flocculation by charge neutralization. When calcium concentration was 12.5 mM,  
30 flocculation was also observed at a pH of 10. Zeta potential remained negative up to  
31 pH 11.5, suggesting that precipitated calcite caused flocculation by a sweeping  
32 coagulation mechanism.

33

34 **Keywords: coagulation, marine algae, enmeshment, biomass, autoflocculation,**  
35 **ballasted flocculation**

36

37

**38 Introduction**

39

40 Microalgae are a promising new source of biomass for production of food, animal  
41 feed, biofuels and feedstocks for the chemical industry (Draaisma et al., 2013; Foley  
42 et al., 2011). Production of microalgal biomass is still very expensive and energy-  
43 intensive and is therefore only economically feasible for high-value products such as  
44 nutritional supplements or fine chemicals (Guedes et al., 2011). The high cost and  
45 energy demand of microalgal biomass production is to a large extent due to the cost of  
46 harvesting (Barros et al., 2015; Show et al., 2013).

47

48 Flocculation is seen as a promising way to reduce the cost and energy inputs of  
49 harvesting of microalgae as it may allow a first separation of the biomass from the  
50 culture medium by simple gravity sedimentation (Benemann et al., 1980; Molina  
51 Grima et al., 2003; Schlesinger et al., 2012). In recent years, several methods for  
52 flocculating microalgae have been developed, including chemical flocculation using  
53 metal coagulants or cationic polymer flocculants , electro-coagulation-flocculation  
54 using aluminum electrodes, microbial flocculation using bacteria or fungi and the use  
55 of magnetic nanoparticles (Vandamme et al., 2013).

56

57 A simple and inexpensive way of inducing flocculation is by spontaneous flocculation  
58 at high pH, which is referred to as autoflocculation or alkaline flocculation (Sukenik  
59 and Shelef, 1984). Studies in the 1980's demonstrated that autoflocculation was  
60 related to precipitation of calcium phosphate (Lavoie and Noüe, 1987; Sukenik et al.,  
61 1985). Autoflocculation by calcium phosphate precipitation, however, only occurs

62 when phosphate concentrations are high ( $> 0.35$  mM), making this method only  
63 useful in phosphate-rich wastewaters (Beuckels et al., 2013).

64

65 When phosphate concentrations are low, autoflocculation can still be induced by  
66 precipitation of calcite (calcium carbonate) and/or brucite (magnesium hydroxide). In  
67 wastewater treatment, this method of flocculation is induced by addition of slaked  
68 lime and is known as lime softening (Ayoub et al., 1986; Elmaleh, 1996). In a  
69 previous study, we elucidated the relative contribution of calcium and magnesium  
70 precipitation to alkaline flocculation for the freshwater microalgae *Chlorella vulgaris*  
71 (Vandamme et al., 2012). Although both calcium and magnesium precipitated during  
72 alkaline flocculation, flocculation could be induced in media containing only  
73 magnesium but not in media containing only calcium. This suggested that, in  
74 freshwater media, alkaline flocculation is induced by precipitation of magnesium  
75 hydroxide or brucite but not by precipitation of calcite.

76

77 Autoflocculation has also been observed in seawater. In seawater, concentrations of  
78 calcium and magnesium are much higher than in freshwater. Therefore, the relative  
79 importance of calcite and brucite in inducing flocculation may be different. The fact  
80 that flocculation starts at a lower pH in seawater than in freshwater suggest that the  
81 underlying mechanism may indeed be different (Sales and Abreu, 2014; Spilling et  
82 al., 2011). Experiments with *Dunaliella* suggested that flocculation at high pH was  
83 mainly related to brucite precipitation (Besson and Guiraud, 2013). Because  
84 *Dunaliella* is grown in hypersaline media, the alkaline flocculation mechanism may  
85 be different in regular seawater media.

86

87 The aim of this study was to understand the role of calcium and magnesium salts in  
88 alkaline flocculation of microalgae in seawater media. Therefore, we combined  
89 precipitation and surface speciation modeling with jar test flocculation experiments  
90 for the model marine microalgae *Phaeodactylum tricorutum*. We elucidated the  
91 relative importance of calcite and brucite by investigating flocculation as a function of  
92 pH in artificial seawater medium containing only calcium, only magnesium or both  
93 cations. Additionally, zeta potential measurements were conducted to study alkaline  
94 flocculation in medium with variable concentrations of calcium and magnesium.  
95  
96

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97 **Materials and methods**

98

99 ***Precipitation model and surface speciation for brucite and calcite in seawater***

100 The concentrations of magnesium (hydroxide) and calcium (carbonate) were  
101 modelled as function of pH in seawater to understand the calcium and magnesium  
102 speciation during alkaline flocculation. The concentrations of total dissolved  
103 magnesium, total dissolved calcium, (bi)carbonate, calcite as  $\text{CaCO}_3 \cdot x\text{H}_2\text{O}(\text{s})$  and  
104 brucite as  $\text{Mg}(\text{OH})_2$  (active) were modelled as functions of pH using Visual Minteq  
105 3.0 (KTH, Department of Sustainable Development, Environmental Science and  
106 Engineering, Stockholm, Sweden). The nominal composition of synthetic seawater  
107 medium (Table 1) was entered using a temperature of 25°C.

108 The surface speciation of brucite and calcite was modeled through simple protonation  
109 and deprotonation reactions described in Brady et al. (2014). Calculations were  
110 carried out using the speciation code PHREEQC (Parkhurst and Appelo, 1999). The  
111 surface site was assumed to be 10 sites  $\text{nm}^{-2}$  (Pokrovsky, 2004). The surface area was  
112 set to  $10^4 \text{ m}^2 \text{ mol}^{-1}$ .

113

114 ***Culturing of microalgae***

115

116 *Phaeodactylum tricoratum* (Pt 86, UGent Belgium) was cultivated in dechlorinated  
117 deionized water to which  $30 \text{ g L}^{-1}$  synthetic sea salt (Homarsel, Zoutman, Belgium)  
118 was added. This synthetic seawater (Table 1) was enriched with inorganic nutrients  
119 according to the concentrations of the Wright's Cryptophyte medium (Guillard and  
120 Lorenzen, 1972). *Chlorella vulgaris* was cultivated in freshwater medium (Table 1).  
121 Both species were cultured in 30 L bubble column photobioreactors that were mixed



122 by sparging with 0.2  $\mu\text{m}$ -filtered air ( $5 \text{ L min}^{-1}$ ). The pH was controlled at 8.5 by  
123 addition of  $\text{CO}_2$ -enriched air (2–3%) using a pH-stat system. Growth of the  
124 microalgae in the photobioreactor was monitored by measuring the absorbance at  
125 750 nm. Flocculation experiments were conducted at the end of the exponential  
126 growth phase when microalgal density was approximately  $0.4 \text{ g L}^{-1}$  dry weight.

127

### 128 *General setup of flocculation experiments*

129

130 Alkaline flocculation was assessed using jar test experiments. 100 mL jars were  
131 stirred using a magnetic stirrer and pH was adjusted between 9 and 12 by addition of  
132 0.5 M sodium hydroxide. The microalgal suspension was mixed intensively  
133 (1000 rpm) for 10 min during and just after pH adjustment, followed by a gentle  
134 mixing (250 rpm) for another 20 min. The suspension was subsequently allowed to  
135 settle for 30 min. The flocculation efficiency was estimated by comparing absorbance  
136 at 750 nm between the pH-adjusted treatment and a control treatment. Samples for  
137 analysis of optical density were collected in the middle of the clarified zone. The  
138 flocculation efficiency  $\eta_a$  was calculated as:

139 
$$\eta_a = \frac{OD_i - OD_f}{OD_i} \text{ (Eq. 1)}$$

140 where  $OD_i$  is the optical density of the control suspension after 30 min sedimentation  
141 without pH adjustment, and  $OD_f$  is the optical density of the suspension after pH  
142 adjustment. Each test was carried out in duplicate.

143

### 144 *The role of magnesium and calcium in flocculation*

145

146 The relative importance of calcium and magnesium in alkaline flocculation was  
147 investigated by testing whether alkaline flocculation could be induced in media with  
148 varying concentrations of calcium or magnesium. *Phaeodactylum* was separated from  
149 the medium using centrifugation at 4,000 g and resuspended in fresh prepared  
150 synthetic seawater medium lacking calcium chloride and magnesium sulphate.  
151 Preliminary tests had shown that *Phaeodactylum* formed a stable suspension after  
152 resuspension in its original medium and that centrifugation and resuspension had no  
153 effect on alkaline flocculation. To investigate the role of calcium chloride in alkaline  
154 flocculation,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  was added in a concentration between 0 and 12.5 mM. To  
155 investigate the role of magnesium, magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) was added in  
156 concentration between 0 and 80 mM. The maximum concentrations used are in the  
157 order of magnitude of the concentrations of these cations occurring in seawater. The  
158 flocculation efficiency of *Phaeodactylum* cells in these synthetic media was assessed  
159 at three pH levels (9.5; 10; 10.5) using jar tests as described above.

160  
161 To evaluate whether magnesium or calcium had precipitated during flocculation, the  
162 concentrations of magnesium and calcium were analyzed after flocculation in the  
163 supernatant and in the biomass pellet using ICP-MS (Agilent 7700x ICP-MS) based  
164 on a method previously used in (Vandamme et al., 2015). In short, supernatant and  
165 biomass pellet were separated using centrifugation at 3,005g and both fractions were  
166 stored at  $-20\text{ }^\circ\text{C}$ . Prior to analysis, samples from the supernatant were diluted and  
167 acidified using nitric acid (70%) to a final concentration of 0.1M. The samples from  
168 the biomass pellet were acidified with 10 ml concentrated  $\text{HNO}_3$  (70 %) and after 48  
169 h the clear solution was diluted 20 times with deionized water.

170

171 Because precipitation of calcite is relatively slow compared to precipitation of  
172 magnesium hydroxide, the influence of additional mixing time was tested for  
173 *Phaeodactylum* resuspended as previously described in synthetic seawater medium  
174 containing 5 mM of bicarbonate and lacking sulfates and magnesium. Jar tests were  
175 conducted as previously described and mixing time was varied from 35 till 95 min in  
176 triplicate. Flocculation efficiency was determined as previous described.

177

#### 178 *Zeta potential measurements*

179

180 The surface charge *Phaeodactylum* cells was assessed by the zeta potential based on  
181 electrophoretic mobility using a Malvern Zetasizer Nano. Zeta potential was  
182 calculated using the Smoluchowski equation. Zeta potential was measured at different  
183 pH levels in media containing either calcium bicarbonate or magnesium sulphate at  
184 concentrations of 7.5 mM. The pH was increased stepwise from 8 to 11.5 using  
185 sodium hydroxide (0.5 M). The zeta potential was evaluated at room temperature in  
186 the electrophoresis cell. For each data point, triplicate cultures were taken for  
187 measurements and for each data, approximately, 25 readings were done. The average  
188 values were then reported.

189 **Results and discussion**

190

191 *Modeling of brucite and calcite precipitation and surface complexation*

192

193 Precipitation of magnesium as brucite (magnesium hydroxide) is predicted to start  
194 when pH exceeds 10. At pH 10.5, residual concentration of  $Mg^{2+}$  in the seawater is  
195 below 40  $\mu M$  (Fig 1A). As brucite precipitation is quadratic in  $OH^-$ , most of the  
196 available  $Mg^{2+}$  is predicted to be precipitated as brucite at  $pH \geq 11$ . The surface  
197 charge of brucite crystals is determined by the pH of the medium. As the isoelectric  
198 point of brucite is 11, the surface charge of brucite precipitates is positive up to a pH  
199 of 11 (Fig 1C). As a result, brucite precipitation should be capable of causing  
200 coagulation of microalgae through charge neutralisation in the pH range 10 to 11.

201

202 Precipitation of calcium as calcite (calcium carbonate) is predicted to start when pH  
203 exceeds 8.5.  $Ca^{2+}$  concentration in seawater is approximately 9 to 12  $\mu M$  while  
204 carbonate concentration is variable but rarely exceeds 5  $\mu M$  in seawaters (Feely et al.,  
205 2004). Therefore, calcite precipitation in seawater is limited by carbonate availability.  
206 As a result, only a fraction of the calcium present in seawater will precipitate as  
207 calcite. Calcite precipitation increases with increasing pH and reaches a maximum at  
208 pH 11 (Fig 1B). Over this pH range, the concentration of carbonate increases as a  
209 result of conversion of bicarbonate to carbonate by increasing pH ( $pK_{a2} = 8.95$  in  
210 seawater 25°C). The surface charge of calcite precipitates is determined by adsorption  
211 of calcium, magnesium, carbonate and sulphates on the surface of calcite crystals  
212 (Brady et al., 2014). The calcite surface in seawater is dominated by sorbed  $Mg$ ,  
213  $>CO_3Mg^+$ , below pH 11, and  $>CO_3^-$  groups at  $pH > 11$  (Fig 1D). When seawater

214 concentrations of calcium and magnesium are present, the overall surface charge is  
215 predicted to be positive at  $\text{pH} < 11$ .

216

217 *Flocculation of Phaeodactylum tricornutum in synthetic seawater medium as a*  
218 *function of pH, magnesium and calcium concentrations.*

219

220 When alkaline flocculation was induced in synthetic seawater containing both  
221 calcium and magnesium, flocculation started as soon as pH was 10 and reached over  
222 90% when pH was 10.5 (Fig 2). This was at a lower pH than in freshwater, where  
223 flocculation occurred at a pH of 11 (Vandamme et al., 2012). Similar results have  
224 been reported in recent studies in experiments with the marine microalgae  
225 *Nannochloropsis*, where flocculation was initiated at a pH of 10 to 10.5 (Sales and  
226 Abreu, 2014; Şirin et al., 2013). At a pH of 10.5, the total dissolved magnesium  
227 concentration remaining in the medium was 70 mM and the total dissolved calcium  
228 concentration was 8.5 mM, which indicates that only 10.5 mM magnesium and 0.6  
229 mM calcium had precipitated during flocculation. The amount of precipitation of both  
230 magnesium and calcium is significantly less than the equilibrium expected (cf. Fig 1).  
231 In general, brucite precipitation is expected to be relatively rapid so that the fact that  
232 equilibrium levels are not reached will motivated further investigation of the effects  
233 of mixing time below (cf. Fig. 5).

234

235 We then evaluated whether alkaline flocculation could be induced in synthetic  
236 seawater lacking either calcium, or magnesium or both (Fig 3). In these experiments,  
237 we also monitored zeta potential to evaluate whether flocculation might be associated  
238 with changes in the surface charge of microalgal cells. No flocculation occurred at

239 any of the pH levels tested when both calcium and magnesium were lacking in the  
240 medium. Also, zeta potential remained constant when pH was increased (Fig 4A).

241

242 In medium lacking calcium, flocculation could be induced when magnesium was  
243 added to the medium. Flocculation efficiencies higher than 80% were observed at the  
244 same pH as in the medium containing both calcium and magnesium (pH 10.5; Fig  
245 3A). When magnesium concentration in the medium was 0.15 mM or higher,  
246 moderate flocculation occurred at pH 10.5 (flocculation efficiency of about 40%).  
247 When magnesium concentration was 7.5 mM or higher, the flocculation efficiency  
248 increased to about 90% at pH 10.5. In medium with 7.5 mM magnesium, the zeta  
249 potential increased from negative to positive values when pH was increased above pH  
250 10.5 (Fig 4B). As predicted by precipitation and surface complexation modeling, our  
251 experimental observations confirm that alkaline flocculation can be induced in  
252 medium containing only magnesium, and that flocculation is at least partly mediated  
253 by charge neutralization. In a previous study, we demonstrated that magnesium  
254 hydroxide precipitation can cause flocculation of the freshwater microalgae *Chlorella*  
255 *vulgaris* (Vandamme et al., 2012). For *Chlorella*, however, a lower magnesium  
256 concentration was required to induce alkaline flocculation than for *Phaeodactylum*. It  
257 is unlikely that this difference is due to differences in biomass concentrations, as  
258 biomass concentrations were comparable for both species (0.4 mg L<sup>-1</sup> in *Chlorella*  
259 versus 0.3 mg L<sup>-1</sup> in *Phaeodactylum*). The observed difference may be due to  
260 differences in cell properties between *Chlorella* and *Phaeodactylum* and/or due to an  
261 effect of the ionic strength of the medium (freshwater versus seawater) (Ayoub et al.,  
262 1986).

263

264 In medium lacking magnesium, flocculation occurred when calcium was added to the  
265 medium. When calcium concentration was 2.5 and 5 mM, flocculation only occurred  
266 at pH 10.5 (Fig 3B). When calcium concentration was 12.5 mM (the normal  
267 concentration in seawater medium) flocculation was also observed at a pH of 10, but  
268 the flocculation efficiency was lower than when pH was 10.5. This is in agreement  
269 with the 50% flocculation efficiency observed at pH 10 when calcium is present in  
270 sufficient concentration in artificial seawater medium (Fig 2). In medium containing  
271 7.5 mM calcium, the zeta potential remained negative up to pH 11.5 (Fig 4C).  
272 Calculations using the results of Brady et al. (2014) as in Fig. 1 suggest that in the  
273 absence of magnesium, the calcite surface remains negative, although the magnitude  
274 of the negative charge varies significantly with the  $\text{Ca}^+/\text{CO}_3^{-2}$  ratio. For the solutions  
275 studied here where calcium concentrations go from 1.25 to 12.5 the reduction in the  
276 calcite surface charge is reduced by more than an order of magnitude, making  
277 interaction between precipitated calcite and the anionic algal surface more favorable.  
278 Our experimental results showed that alkaline flocculation can also be induced in  
279 water containing calcium but lacking magnesium. Flocculation must have been due to  
280 calcite precipitation and not calcium phosphate precipitation as phosphate  
281 concentrations in the medium were very low ( $<0.05$  mM). While our model predicted  
282 that calcite precipitation is initiated at pH 8.5, our experiments showed that  
283 flocculation only occurred at a pH of 10, or an even higher pH when calcium  
284 concentrations were lower than those typically occurring in seawater. This  
285 discrepancy could be related to kinetic aspects of calcite formation, which is not  
286 included in our model (Shaojun and Mucci, 1993). Therefore we additionally tested  
287 the influence of mixing time on flocculation efficiency as a function of pH in  
288 synthetic seawater containing 5 mM of bicarbonate and lacking magnesium and

289 sulfates (Fig 5). At pH 8, no flocculation was observed even for 95 min of mixing. At  
290 pH 9, flocculation efficiency was 34% for 35 min of mixing and increased up to 70%  
291 for 50 min of mixing. This increase in flocculation efficiency at pH 9 is now in  
292 correspondence with the predictions for calcite precipitations (Fig 1B). This  
293 additional experiment showed that in contrast to brucite, calcite mediated flocculation  
294 requires longer mixing than 30 minutes, which is traditionally used in jar tests  
295 (Bratby, 2006).

296

### 297 ***Relative importance of calcite and brucite***

298

299 When pH of *Phaeodactylum* cultures in synthetic seawater medium is increased,  
300 alkaline flocculation occurred at a pH of 10 -10.5 (Fig 2). In this pH window,  
301 precipitation of both calcite and brucite may contribute to flocculation. Theoretically,  
302 calcite could precipitate at a lower pH of 8.5 but it was demonstrated that longer  
303 mixing was needed to obtain satisfactory flocculation in relation to precipitation  
304 model (Fig 5). In outdoor microalgal cultures, pH increases gradually due to  
305 photosynthetic activity and pH variation is stabilized by supply of carbon dioxide.  
306 Due to exchange with the atmosphere and conversion of carbon dioxide to carbonates,  
307 calcite concentrations may slowly increase and induce flocculation. Measurements of  
308 zeta potential indicated that alkaline flocculation of *Phaeodactylum* in medium  
309 lacking magnesium was not associated with a reversal of the surface charge (Fig 4C).  
310 This suggests that calcite precipitates were either uncharged or negatively charged  
311 and flocculation occurred through a sweeping mechanism rather than charge  
312 neutralization. The surface charge of calcite crystals, however, depends on the  
313 presence of both calcium and magnesium in solution (Brady et al. 2014). The medium



314 that was used in our experiment for Figs. 4C and 5 lacked magnesium, and in the  
315 absence of magnesium the calcite surface charge is expected to be negative; similar  
316 behavior is seen in Fig. 1D for pH values where magnesium precipitates as brucite.  
317 As discussed around Fig. 1D, the calcite surface charge is a balance between sorption  
318 of cations as  $> \text{CO}_3\text{Ca}^+$  and  $> \text{CO}_3\text{Mg}^+$  versus  $> \text{CO}_3^-$  surface groups, and the lack of  
319 Mg shifts the balance to negative.

320

321 According to our experiments, flocculation by precipitation of brucite occurred in a  
322 very narrow pH interval of 10 to 10.5. This is a consequence of the strong  
323 dependence of brucite precipitation on  $\text{OH}^-$  as well as the relatively rapid kinetics  
324 associated with brucite precipitation. As a pH of 10.5 is near the limit of the process  
325 of bicarbonate utilization in photosynthesis (Prins and Elzenga, 1989), addition of  
326 base may be required to induce flocculation by brucite precipitation; however, this  
327 might be mitigated by the buffering associated with brucite precipitation. The  
328 magnesium hydroxide that is formed is positively charged and will cause immediate  
329 flocculation through charge neutralization and possibly also through a sweeping  
330 mechanism. Although the high ionic strength of seawater results in higher magnesium  
331 requirements to induce flocculation when compared to freshwater, this is not a  
332 limiting factor due to the high magnesium concentration in seawater. This makes  
333 brucite mediated flocculation an easier process to control compared to calcite  
334 mediated flocculation.

335

336 Ayoub et al. 1986 concluded that seawater induced microalgal flocculation was  
337 independent of microalgal biomass concentration, while in freshwater conditions  
338 there was a linear behavior reported between magnesium and biomass concentration

339 for alkaline flocculation associated with brucite precipitation (García-Pérez et al.,  
340 2014). However, present results in marine conditions showed that brucite-mediated  
341 flocculation was mainly caused by charge neutralization. By this, one could expect a  
342 linear behavior of Mg dosage with biomass concentration which is in line with the  
343 observation made by García-Pérez et al. (2014). While for calcite, one could expect a  
344 independent behavior between calcium dosage and biomass concentration because  
345 calcite-mediated flocculation was mainly caused by sweeping, which is in line with  
346 the conclusions made by Ayoub et al. 1986. This suggests that flocculation conditions  
347 and process strategy could be adapted based on the initial biomass concentration. The  
348 effect of microalgae biomass concentration on the flocculation efficiency, pH,  
349 calcium and magnesium concentration in marine conditions deserves therefore  
350 further detailed study.

351

## 352 **Conclusions**

353

354 This study demonstrates that alkaline flocculation can be induced in a narrow pH  
355 range of 10-10.5 for the marine diatom *Phaeodactylum tricornutum* by the  
356 precipitation of brucite. A higher dosage of magnesium was needed to obtain similar  
357 flocculation efficiency compared to freshwater conditions. Alkaline flocculation was  
358 also induced in medium lacking magnesium. The precipitation of calcite was slower  
359 compared to brucite but it caused alkaline flocculation as well. This process was  
360 based on a sweeping mechanism, while brucite mainly caused alkaline flocculation  
361 based on a charge neutralization mechanism.

362

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364

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380 **References**

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ACCEPTED MANUSCRIPT

460 **Figure Captions**

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462

463 **Fig 1(A) Concentration of total dissolved magnesium (as  $Mg^{2+}$ ) and precipitated**464 **brucite (as  $Mg(OH)_2$  (active)); (B) total dissolved calcium (as  $Ca^{2+}$ ), bicarbonate**465 **(as  $HCO_3^-$ ) carbonate (as  $CO_3^{2-}$ ) and precipitated calcite (as  $CaCO_3 \cdot xH_2O$  (s)**466 **modelled as function of pH in artificial seawater. (C) Brucite and (D) calcite**467 **surface speciation modelled as function of pH in artificial seawater.**

468

469 **Fig 2 Flocculation efficiency of *Phaeodactylum tricornutum* in synthetic media as**470 **function of pH (n = 2, 1 $\sigma$ )**

471

472 **Fig 3 Flocculation efficiency of *Phaeodactylum tricornutum* cells resuspended in**473 **(A) synthetic seawater lacking calcium with varying concentrations of**474 **magnesium and (B) lacking magnesium with varying concentrations of calcium**475 **at pH levels 9.5, 10 and 10.5 (n = 2, 1 $\sigma$ )**

476

477 **Fig 4 Zeta potential as function of pH for resuspended *Phaeodactylum***478 ***tricornutum* (A) lacking calcium and magnesium, (B) with 7.5 mM magnesium**479 **sulphate and (C) with 7.5 mM calcium bicarbonate (n = 3, 1 $\sigma$ )**

480

481 **Fig 5 Influence of mixing time on flocculation efficiency for *Phaeodactylum***482 **resuspended in synthetic seawater containing 5mM of bicarbonate lacking**483 **magnesium and sulphates at pH 8,9,10 and 11 (n = 2, 1 $\sigma$ )**

484

**Table 1. Concentrations of the main ions in the medium used to culture *Phaeodactylum tricornutum* (synthetic seawater) in comparison with Wright's Cryptophyte freshwater medium used for *Chlorella vulgaris***

	Synthetic seawater (mM)	Freshwater (mM)
Cl <sup>-</sup>	442.1	1.7
Na <sup>+</sup>	338.6	1.9
Mg <sup>2+</sup>	80.5	1.0
Ca <sup>2+</sup>	9.1	2.7
K <sup>+</sup>	6.4	0.3
HCO <sub>3</sub> <sup>-</sup>	5.0	0.15
SO <sub>4</sub> <sup>2-</sup>	40.2	1.3
Conductivity (mS cm <sup>-1</sup> )	43.0	0.8



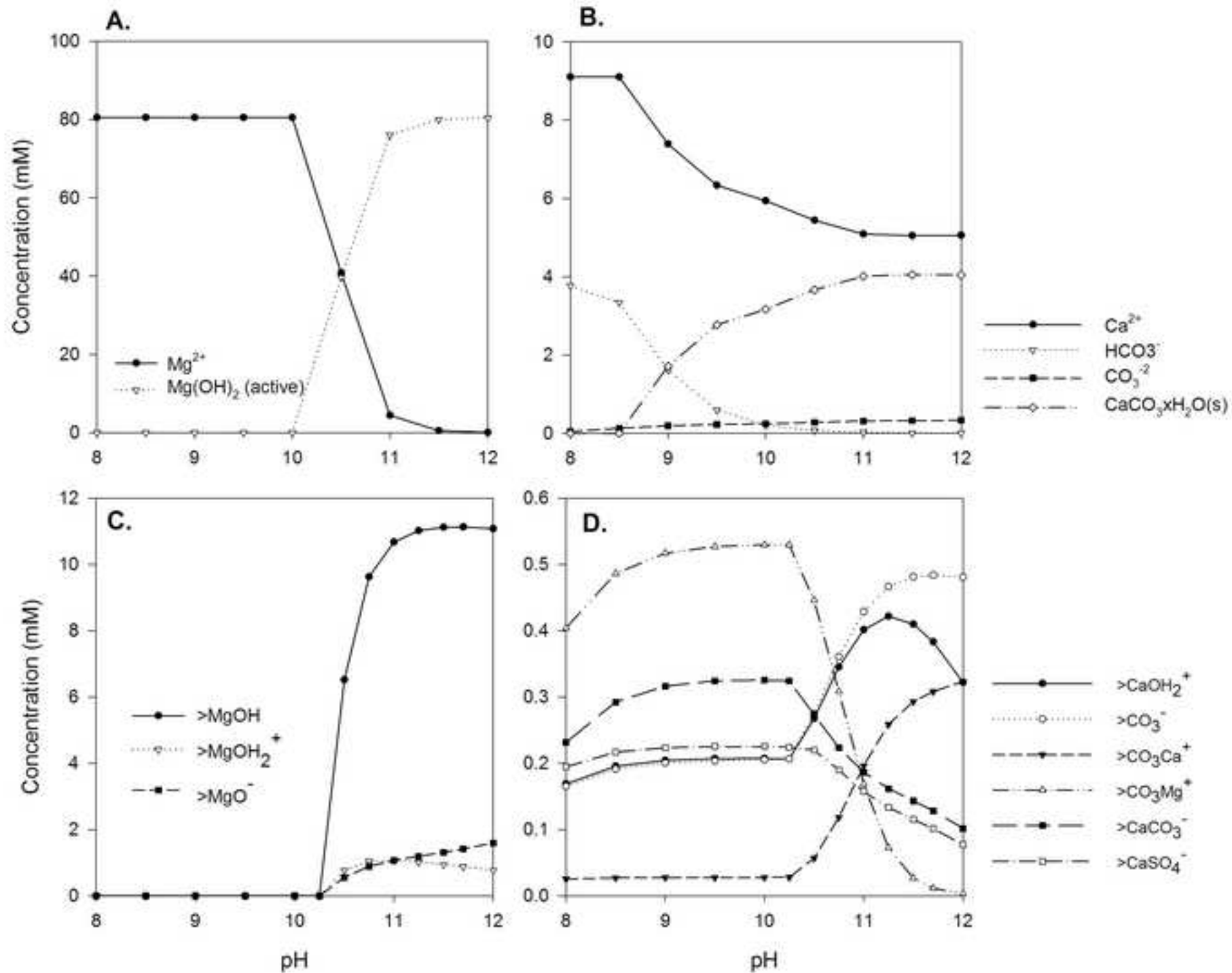
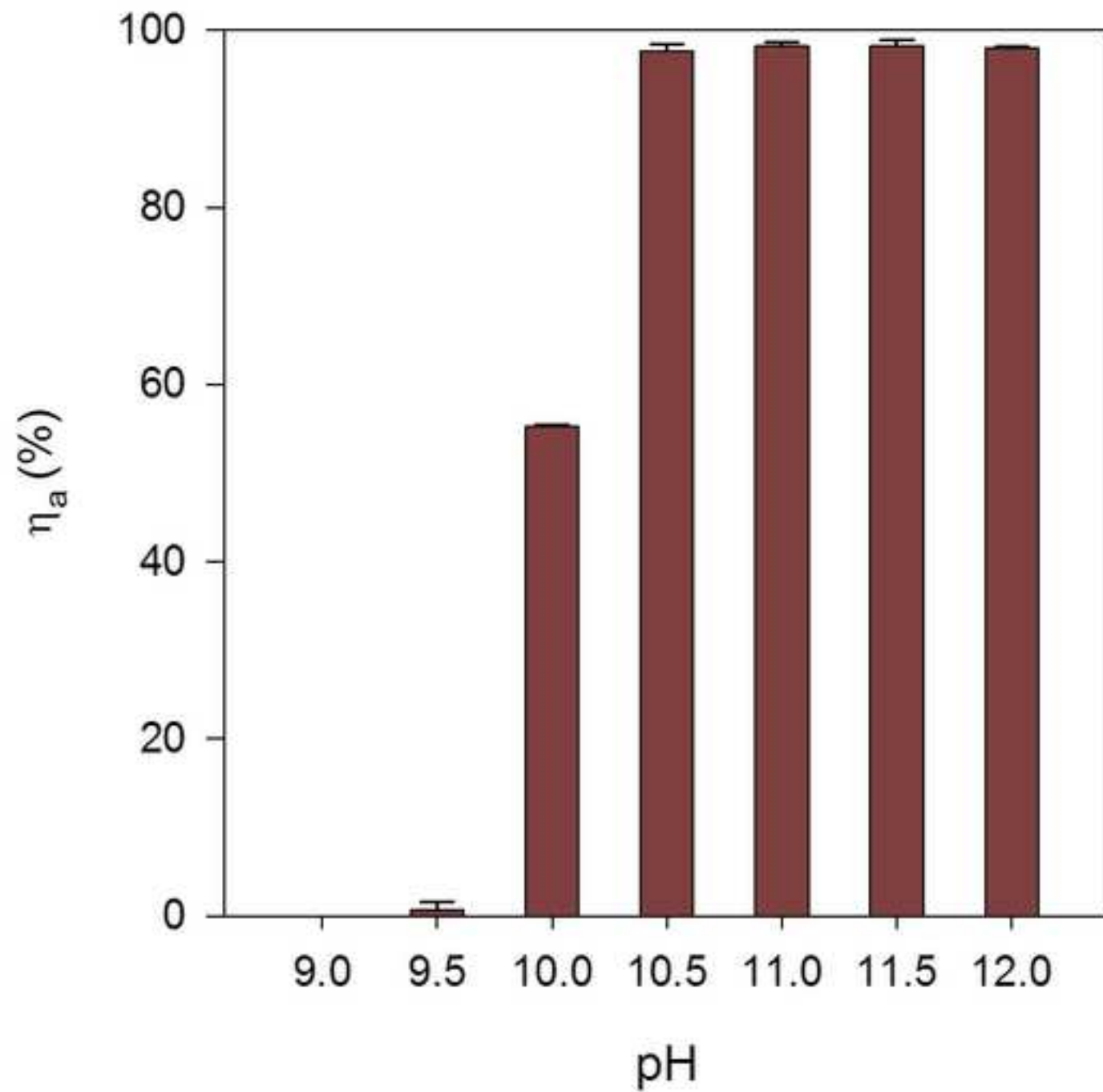
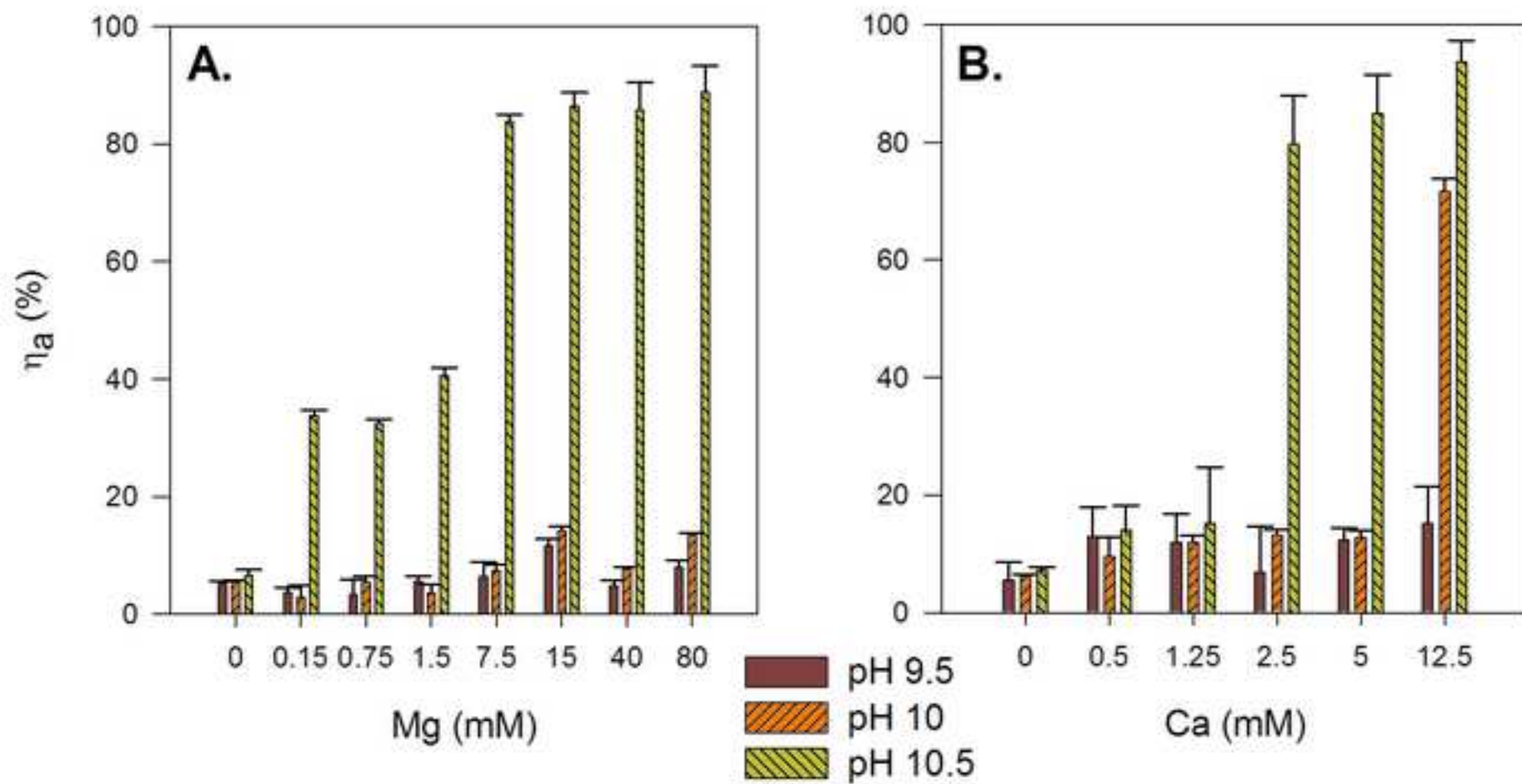
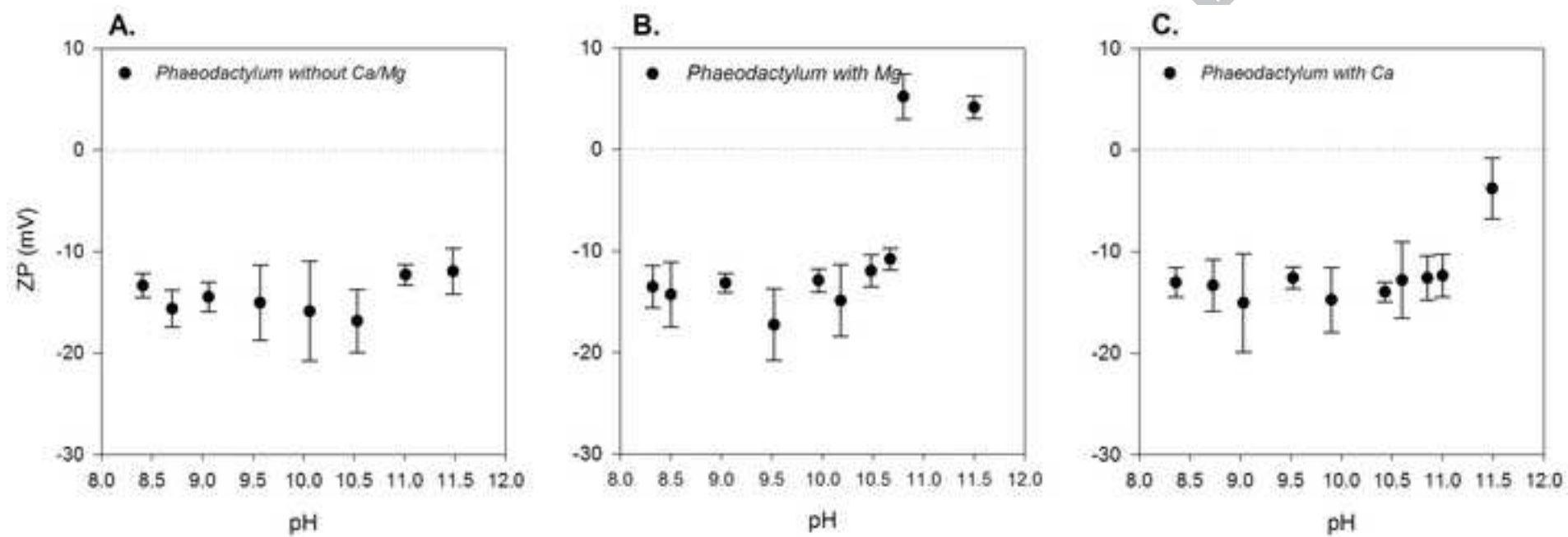
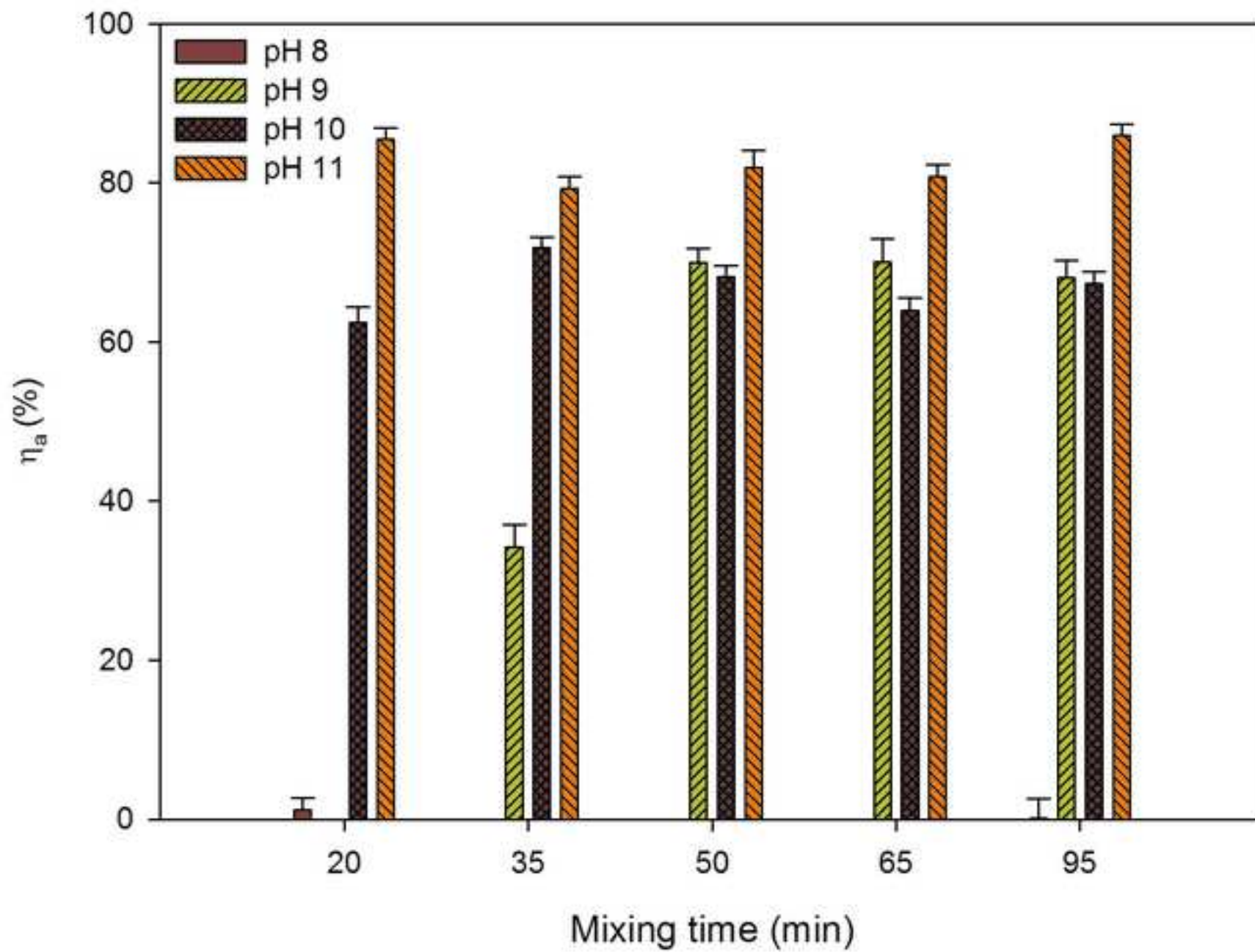


Figure 2









**Highlights**

- Both brucite and calcite are involved in alkaline flocculation in marine conditions
- Flocculation efficiency reached 90% at pH 10.5 consistent with precipitation models
- Mg precipitated as brucite caused flocculation dominantly by charge neutralization
- Ca precipitated as calcite caused flocculation dominantly by sweeping flocculation