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2 **Reversible flocculation of microalgae using magnesium hydroxide**

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Abstract

Flocculation of microalgae is a promising low-cost strategy to harvest microalgae for bulk biomass production. However, residual flocculants can interfere in further downstream processes or influence biomass quality. In this study, a new concept is demonstrated based on reversible magnesium hydroxide flocculation, using *Chlorella vulgaris* and *Phaeodactylum tricornutum* as respectively a freshwater and a marine model species. We show that flocculation was induced by precipitation of magnesium hydroxide at high pH (10 to 10.5). This resulted in a magnesium content of the microalgal biomass of 5% for *Chlorella* and 18% for *Phaeodactylum*. After pre-concentration of the microalgal biomass by gravity sedimentation, 95% of the precipitated magnesium hydroxide could be removed from the biomass by mild acidification (pH 7 to 8). The pH fluctuations experienced by the microalgae during flocculation/de-flocculation had no influence on biomass composition (FAME, total N and P, carbohydrates, proteins, mineral content) and on the viability of microalgal cells. Magnesium can thus be used as pH-dependent reversible flocculant for harvesting microalgae in both marine and freshwater medium.

Introduction

Microalgae are a highly promising feedstock for production of biofuels. These unicellular micro-organisms have a high areal productivity and produce biomass that is low in structural compounds like cellulose or lignin and can thus be almost entirely valorised in a biorefinery context, with the lipid fraction of the biomass being used for biodiesel production while the remaining protein-rich residue can be used as animal feed [1]. Moreover, they do not compete directly with food production by using agricultural crop area. Production of microalgae, however, is a relatively energy-intensive process. To make microalgae competitive with conventional agricultural crops, the cost and energy inputs of the production process need to be reduced by at least an order of magnitude. One of the major challenges is situated in the harvesting of the microalgae. Because microalgae cells are small (5-50 µm) and the biomass concentration in the medium is low in case of open pond cultivation systems (< 1 g dry biomass L^{-1}), harvesting using centrifugation or membrane filtration is too costly and energy-intensive[1, 2].

It is widely believed that flocculation has a lot of potential to reduce the cost and energy demand of microalgae harvesting [3–6]. Using flocculation, microalgae can be pre-concentrated 20-50 times using simple gravity sedimentation or flotation. The pre-concentrated biomass can then subsequently be further dewatered using a mechanical method such as filtration or centrifugation [7]. Because the bulk of the water has been removed during the flocculation step, the cost and energy demand for further dewatering using mechanical methods is reduced by more than an order of magnitude[3]. This has made microalgae flocculation an active field of research in the past years. Many flocculation technologies that have been widely used in other industries have been applied to microalgae harvesting. These

include metal coagulants, synthetic or natural polymer flocculants, electro-coagulation-flocculation or lime softening [8, 9]. The main disadvantage of most flocculation technologies, however, is that the harvested biomass becomes contaminated with the flocculant, which may interfere with downstream processing. Borges et al., for instance, showed that anionic polyacrylamides significantly influenced the fatty acid profile of lipid extracts from marine microalgae [10]. Biomass contamination with a flocculant may also interfere with valorisation of the protein-rich biomass residue remaining after extraction of biofuels as animal feed.

When flocculation is used for harvesting microalgae it is part of a two-stage harvesting process. During the first stage, flocculation is used in combination with gravity sedimentation or flotation to pre-concentrate the biomass. During the second stage, the pre-concentrated biomass is completely dewatered using a mechanical method like centrifugation. The ideal flocculant for microalgae harvesting is a reversible flocculant that can be removed from the microalgae biomass after the first stage, i.e. after pre-concentration of the biomass by sedimentation or flotation. Magnetic iron oxide nanoparticles have been proposed for reversible flocculation of microalgae. These nanoparticles are functionalized with a pH-reversible surface charge. They interact with microalgae cells and cause flocculation at pH 12 but can be recovered multiple cycles from the pre-concentrated microalgae biomass at pH 2 [11]. The cost of commercially available magnetic nanoparticles is today high, it may become a promising tool for harvesting microalgae when low-cost alternatives become available [12, 13]. Here, we propose an alternative method for harvesting microalgae using reversible flocculation based on magnesium hydroxide.

One of the most promising low-cost methods for harvesting microalgae that has emerged in the past years is pH-induced flocculation or the spontaneous flocculation of microalgae at high pH. pH-induced flocculation is the result of precipitation of calcium or magnesium salts. Calcium phosphate, for instance, precipitates at pH levels of 9 or above and can cause effective flocculation of microalgae. The disadvantage of flocculation by calcium phosphate precipitates is the high concentration of phosphate that is required. Indeed, more phosphate is required for flocculating the biomass than for producing the microalgae biomass [14]. Magnesium hydroxide precipitates at a pH of 10-11 and can cause flocculation of microalgae through charge neutralisation and/or by a sweeping mechanism. As most waters contain sufficient quantities of magnesium, the only cost involved in magnesium hydroxide flocculation is the cost of base required to increase the pH. This cost is low (about 18 $ ton dry biomass^{-1} harvested) if a low-cost base such as lime is used [15]. Several recent studies have highlighted the potential of pH-induced flocculation induced by magnesium hydroxide precipitation as a low-cost harvesting method [3, 16, 17]. Nevertheless, this method of harvesting results in a contamination of the biomass with magnesium hydroxide. Although magnesium hydroxide is non-toxic, it is nevertheless desirable to remove it from the harvested biomass.

Magnesium hydroxide precipitates above a pH of 10 to 11 (depending on the magnesium concentration in the medium) and dissolves below this pH [18]. Magnesium hydroxide can therefore theoretically be removed from the flocculated and pre-concentrated microalgae biomass by means of acidification. In this way, magnesium hydroxide could be used as a reversible flocculant: at high pH precipitation of magnesium hydroxide causes flocculation while at low pH the magnesium hydroxide dissolves and can be removed from the biomass. Whether this approach is applicable in practice depends on the pH required to dissolve the

magnesium hydroxide as well as the rate of dissolution during de-flocculation. If the pH required is too low and/or the process takes too long, this approach is not feasible. Moreover, the large pH fluctuations that are involved in the process may influence the quality in terms of composition of the harvested microalgae biomass.

In this study, we evaluated the use of magnesium hydroxide as a reversible flocculant for harvesting microalgae. We determined the optimal pH for flocculation and de-flocculation and evaluated the impact of this reversible flocculation process on the microalgae biomass composition and viability of the microalgae cells. Because magnesium concentrations differ by almost two orders of magnitude between freshwater and seawater, the use of magnesium hydroxide as a reversible flocculant was tested for a freshwater (*Chlorella vulgaris*) as well as a marine (*Phaeodactylum tricornutum*) model microalgae species.

Materials and Methods

Cultivation of C. vulgaris and P. tricornutum

The freshwater green microalgae *Chlorella vulgaris* 211-11b (SAG) and the marine diatom *Phaeodactylum tricornutum* 1055/1 (CCAP) were used as model species for studying reversible magnesium flocculation. Both species have previously been used as a model species in pH-induced flocculation studies [15, 19].

C. vulgaris and P. tricornutum were both cultured in batch mode in 10 L plexiglass bubble column photobioreactors (20 cm diameter) using modified Wright's Cryptophyte medium (Table 1) [20]. Additional magnesium and calcium were added to the cultivation medium of both species in concentrations that are typically found in surface and in marine waters. The reactors were aerated with 0.2 μm filtered air (5 L min^{-1}) and pH was maintained at 8.5 through pH-controlled addition of carbon dioxide to the air flow. The culture was irradiated from two sides with daylight fluorescent tubes (Osram Grolux Sylvania, Germany), giving a photon flux of 60 μmol m^{-2} s^{-1} at the surface of the reactor. Algal biomass was monitored by measuring absorbance at 750 nm. Absorbance measurements were calibrated against dry weight, which was in its turn determined gravimetrically on pre-weighed GF/F glass fiber filters [21]. Flocculation experiments were carried out at the end of the exponential growth phase at day 6 of cultivation.

Flocculation experiments

We first determined the degree of contamination of the microalgae biomass by magnesium at different pH levels during pH-induced flocculation by magnesium hydroxide. Flocculation

experiments were carried out in jars containing 100 mL of microalgae broth and mixing was achieved by magnetic stirring. pH was adjusted by addition of 0.5 M NaOH in concentrations ranging from 0 to 4mM . NaOH was dosed during 10 min of intensive mixing at 1000 rpm. Then, the suspensions were mixed gently (250 rpm) for another 20 min while pH of each treatment was monitored, followed by 30 min of settling. The flocculation efficiency was calculated based on changes in the optical density (measured at 750 nm) prior to pH adjustment (OD_i) and after settling (OD_f). The flocculation efficiency, or the percentage of microalgae biomass removed from suspension, was calculated as:

$$\text{Microalgae flocculation efficiency} = \frac{OD_i - OD_f}{OD_i}$$

To quantify the amount of magnesium that had precipitated during pH-induced flocculation, we compared concentrations of magnesium in the supernatant and in the biomass pellet after flocculation. Subsamples from supernatant and biomass pellet were centrifuged at 3,005 g and stored at -20°C.

Samples from the supernatant were diluted and acidified to obtain a final concentration of 0.1 M HNO_3 (addition of 0.1 mL 5M HNO_3 to a 5 mL sample). The wet samples from the biomass pellet were acidified with 10ml concentrated HNO_3 (70%) and after 48 hours the clear solution was diluted 20 times with deionized water. Magnesium concentrations were measured using ICP-MS (Agilent 7700x ICP-MS).

De-flocculation experiments

To evaluate the amount of magnesium that could be redissolved after acidification, triplicate 1 L suspensions of microalgae were flocculated. Based on previous results, the optimal dosage of base in order to maximize flocculation efficiency and minimize the magnesium

content in the biomass was 4 mM of NaOH for *Chlorella* and 5 mM of NaOH for *Phaeodactylum*. After 20 min of stirring at 250 rpm, the suspension was allowed to settle 30 min in 1 L Imhoff cones and the settled biomass pellet and the supernatant were separated by decantation. 50 mL of the biomass pellet was subsequently de-flocculated by acidification with 5 M HCl until pH 6, 7 and 8 and continuously stirred at 250 rpm for 30 and 60 minutes. Subsamples were taken and prepared as previously described to compare magnesium concentrations in supernatant and biomass pellet before, after flocculation and after de-flocculation.

Impact of flocculation and de-flocculation on the composition of the harvested biomass

Flocculation (same conditions as above) and de-flocculation (pH 8 for 30 min) was carried out on triplicate 1 L suspensions in order to evaluate the influence of the process on the composition of the harvested biomass. After flocculation and de-flocculation, the biomass pellet was separated from the supernatant by centrifugation, freeze-dried and stored at -80°C until further analysis. Because the increase in pH to induce flocculation may not only result in precipitation of magnesium hydroxide but also of other minerals (e.g. calcium phosphates, calcium carbonate or calcium ammonium phosphate), we determined the content of magnesium, calcium and phosphorus using ICP-MS as previously described. Carbohydrates, fatty acid methyl ester (FAME) and protein content were compared with biomass before flocculation as a control treatment to evaluate the influence of flocculation de-flocculation on the biochemical composition of the biomass. The carbohydrates were extracted and measured using the phenol-sulphuric acid method [22, 23]. FAME content was analysed using a direct transesterification method [24] and the obtained FAMEs were separated by gas chromatography (GC) with cold on-column injection and flame ionisation detection (FID)

(Thermo Scientific Trace GC Ultra) using a Grace EC Wax column (length: 30 m, ID 0.32 mm, film: 0.25 µm). The used time–temperature program was: 70–180 °C (5 °C/min), 180–235 °C (2 °C/min), 235 °C (9.5 min). Fatty acid identification was performed using standards containing a total of 35 different FAMEs (Nu-check). Peak areas were quantified with Chromcard for Windows software (Interscience). For analysis of the nitrogen content, the biomass was digested using an alkaline persulphate digestion [25]. The nitrogen content in the digestate was measured as nitrate and using Hach-Lange standard kits LCK 339 (Merck, Darmstadt, Germany). The protein content of the biomass was calculated by multiplying the nitrogen content (% on dry weight) with the general 6.25 conversion factor [26]. Results were statistically analysed using a one-sample t-test with a level of significance of 0.05 (Sigmaplot Systat Software).

Cell viability assessments

The effect of pH shifts on cell viability was first studied in a cultivation experiment using modified Wright's Cryptophyte medium (Table 1). Deflocculated biomass (60 min at pH 8) was compared to a control inoculum both for *Chlorella* and *Phaeodactylum.* Biomass density was monitored daily spectrophotometrically (750nm) for 7 days.

Secondly, the maximum quantum yield of photosystem II (ratio of variable versus maximal fluorescence; Fv:Fm) was measured to evaluate the influence of harvesting by flocculation/de-flocculation on the photosynthetic apparatus using an AquaPEN PAM fluorometer (Photon Systems Instruments). This parameter is a sensitive indicator of stress experienced by microalgae and is often used for evaluating toxicity of substances towards microalgae [27]. Quantum yield (n=3) was compared for *Chlorella* and *Phaeodactylum* before

flocculation, after 30 min of sedimentation and after de-flocculation (60 min at pH 8). Measurements were conducted after 20 min of dark adaptation of the microalgae. Cells treated with 15% H_2O_2 for 30 min were used as negative control. Results were statistically analysed using a two way ANOVA and a pairwise multiple comparison (Tukey Test) with a level of significance of 0.05 (Sigmaplot Systat Software).

Finally, cells were stained using Evan's Blue for *Chlorella* and *Phaeodactylum* before flocculation, after 30 min of sedimentation and after de-flocculation (60 min at pH 8). This was compared to a positive (no treatment) and a negative control (15% H_2O_2 for 30 min). Cells were examined using light microscopy (Olympus BX 51).

Results and discussion

Magnesium precipitation during flocculation

We first experimentally quantified the degree of contamination of the biomass by magnesium during pH-induced flocculation of *Chlorella* and *Phaeodactylum* (Fig 1). The biomass concentration was about 0.1 g L^{-1} in the *Chlorella* culture and 0.5 g L^{-1} in the *Phaeodactylum* culture.

In both species, flocculation occurred after addition of about 1 mM sodium hydroxide. At the onset of flocculation, the pH was 10.7 for *Chlorella* and 10.3 for *Phaeodactylum*. In the *Chlorella* experiment, about 0.25 mM of the initial 1 mM magnesium had precipitated from solution at the onset of flocculation. Continued addition of sodium hydroxide did not change the flocculation efficiency but resulted in an increased precipitation of magnesium until all magnesium was removed from solution (Fig 1c). In the *Phaeodactylum* experiment, about 1.2 mM of the initial 50.15 mM magnesium had precipitated from solution at the onset of flocculation (result not shown). Increasing the dose of sodium hydroxide resulted in increased precipitation of magnesium, up to 2.5 mM magnesium at a dosage of 4 mM sodium hydroxide (Fig 1f). This massive precipitation of magnesium resulted in the formation of a large amount of white sludge. The maximum flocculation efficiency was 90% in *Chlorella* but only 73% in *Phaeodactylum*. The flocculation efficiency in *Phaeodactylum* was probably underestimated due to the formation of a fine white precipitate during flocculation, most likely calcium carbonate. This resulted in a high residual absorbance of the supernatant despite the fact that most cells had clearly flocculated.

We can assume that magnesium precipitated as magnesium hydroxide at high pH [15, 28]. Magnesium hydroxide is positively charged (point of zero charge 11.5) and can cause flocculation through charge neutralisation and/or by a sweeping flocculation mechanism. The flocculation mechanism is in that perspective similar to flocculation caused by aluminium sulphate or ferric chloride, which also form positively charged hydroxides when dissolved in water [29]. At the onset of flocculation, the concentration of magnesium in the microalgae biomass was 4.7 % for *Chlorella* and 5.8 % for *Phaeodactylum* (Fig 1c and 1f), but this increased rapidly as when the dosage of sodium hydroxide increased. This magnesium hydroxide would end up in the harvested biomass and would result in a very high total mineral content of the harvested biomass. In the current commercial production of microalgae, guidelines state that the total mineral content should remain below 10 % [30]. With a magnesium content of approximately 5 %, the magnesium hydroxide content alone will already result in a mineral content higher than 10 %.

Dissolution of precipitated magnesium after de-flocculation

We subsequently evaluated whether the precipitated magnesium hydroxide could be removed from the flocculated biomass by means of de-flocculation by mild acidification. In this experiment, the biomass concentration was 0.30 g L^{-1} for *Chlorella* and 0.35 g L^{-1} for *Phaeodactylum*.

Prior to pH-induced flocculation, a magnesium concentration of only 0.6 % for *Chlorella* and 1.26 % for *Phaeodactylum* was found in the biomass pellet (Fig 2, BF). Magnesium in microalgae is mostly associated with chlorophylls, with ATP and with enzymes involved in phosphate metabolisms (ATP, nucleic acids) and typically amounts to a magnesium content of

about 0.5 %, which is close to what we measured in *Chlorella* [31, 32]. The higher magnesium content of *Phaeodactylum* may be due to the fact that some magnesium hydroxide had precipitated during cultivation as a result of the high pH of the cultures. Due to the higher magnesium concentration in seawater when compared to freshwater, magnesium precipitation is more likely to occur in seawater than in freshwater medium.

After addition of sodium hydroxide to induce flocculation, the amount of magnesium in the biomass pellet increased substantially to 5 % in *Chlorella* and 18 % in *Phaeodactylum* (Fig 2, AF). Magnesium concentration in the medium decreased from 1 mM before to 0.48 mM after flocculation in the *Chlorella* experiment, indicating that almost half of the magnesium had precipitated from solution. Because of the extremely high concentration of magnesium in the seawater medium, pH-induced flocculation had no measurable effect on the magnesium concentration in the medium in the *Phaeodactylum* experiment.

The flocculated microalgae were separated from the culture medium and acidified to re-dissolve the magnesium that had precipitated during pH-induced flocculation. Three pH levels were tested (6, 7 and 8) and the quantity of magnesium in the solution as well as in the biomass pellet was measured after 30 and 60 minutes (Fig. 2). For the pH 6 and 7 treatments in *Chlorella*, the magnesium in the biomass pellet after de-flocculation was the same as before pH-induced flocculation, indicating that all magnesium that had precipitated was effectively re-dissolved. For the pH 8 treatment, the magnesium content of the biomass pellet was slightly higher than the initial magnesium content (0.8% instead of 0.6%). Nevertheless, this indicates that 95% of the magnesium that had precipitated during pH-induced flocculation could be re-dissolved by de-flocculation to pH 8. The de-flocculation time (30 or 60 minutes) had no effect on the magnesium content in the biomass in the *Chlorella* experiment. For

Phaeodactylum, the magnesium content of the biomass pellet was reduced to 0.9% at pH 6, 1.1% at pH 7 and 1.5% at pH 8. The magnesium content was slightly lower after 60 than after 30 minutes of de-flocculation. The fact that the magnesium concentration in the biomass pellet was in some cases lower than before pH-induced flocculation may be due to the fact that acidification removed magnesium that had already precipitated in the *Phaeodactylum* culture prior to flocculation.

These results indicate that the magnesium that precipitates during flocculation at high pH can easily be re-dissolved and removed from the harvested biomass. Even a mild de-flocculation by acidification at pH 8 for 30 minutes is sufficient to remove 95% of the magnesium from the flocculated and pre-concentrated biomass. Magnesium hydroxide is a mineral that is easily dissolved. Moreover, the ionic composition of the medium or the presence of organic compounds in the medium has little effect on the dissolution kinetics of magnesium hydroxide[33]. To acidify the medium to pH 8, only 0.75 mmol of hydrochloric acid was added for *Chlorella* and 2.35 mmol for *Phaeodactylum*.

Influence of flocculation – de-flocculation on biomass quality

The rapid pH shifts experienced by microalgae during pH-induced flocculation and de-flocculation may influence the viability of the microalgae cells as well as the biochemical composition of the microalgae biomass.

Although it is not essential that microalgal cells remain viable during harvesting, viability indicates that cell integrity remains intact and this in turn implies that the cell content is not released into the medium during harvesting. In our experiments, cell viability did not appear

to be affected by the pH shifts. When microalgae cells were flocculated at pH 10.9 and magnesium hydroxide was re-dissolved at pH 8 for 60 min., the cells immediately resumed growth when transferred to fresh culture medium (Fig 3). For both species, growth rates were comparable between the flocculated cells and the control cells. The quantum yield of photosystem II of the cells or Fv:Fm, which is a sensitive indicator of stress in plants and microalgae [27], did not significantly differ before flocculation, immediately after flocculation and after re-dissolution of magnesium ($P > 0.270$), whereas a positive control consisting of hydrogen peroxide treatment resulted in a large and significant decrease in Fv:Fm ($P < 0.001$) (Fig 4). Finally, Evan's Blue staining showed that the cell membrane remained intact throughout the flocculation – de-flocculation treatment in both *Chlorella* and *Phaeodactylum* (Fig 5), while H_2O_2 treated cells again showed a clear blue staining of the cytoplasm indicating a decrease of cell membrane integrity (Fig 5 (B,E)).

The carbohydrate, FAME, protein content of the biomass was also compared before flocculation and after a flocculation – de-flocculation treatment (Table 2). In general, no significant differences were observed. The pH increase required to induce precipitation of magnesium hydroxide may also result in precipitation of other minerals from solution, such as calcium phosphate and calcium carbonate[18]. These minerals may dissolve less easily during de-flocculation by acidification and may thus accumulate in the harvested biomass. Calcium, magnesium and phosphorus concentrations in the biomass were compared before and after the flocculation – de-flocculation treatment (Table 2). We did not observe an significant increase in mineral concentration of any of these minerals. We did, however, observe a small but significant decrease in the calcium content for *Chlorella* (Table 2, $P = 0.008$). Possibly, some precipitation of calcium carbonate had occurred in the cultures as a result of photosynthetic

increase in pH and this mineral was dissolved during de-flocculation of the pre-concentrated biomass.

Magnesium hydroxide as a reversible flocculant for freshwater as opposed to marine microalgae

Our results show that magnesium hydroxide can be used as a reversible flocculant for both freshwater and marine microalgae. Addition of base causes precipitation of magnesium hydroxide and this acts as an effective flocculant. Previous work has shown that the biomass can be pre-concentrated about 50 times using magnesium hydroxide flocculation [28]. After flocculation and pre-concentration of the biomass by gravity sedimentation, the precipitated magnesium hydroxide can be removed from the pre-concentrated biomass by means of de-flocculation by mild acidification. For both species, a decrease in pH to 8 for 60 min was sufficient to remove 95% of the precipitated magnesium from the biomass.

Freshwaters have a relatively low concentration of magnesium, ranging from 0.1 mM in soft water to 1 mM in hard water. Because the magnesium content of freshwater is low, the pH has to be raised to a relatively high level before flocculation can be induced (pH 10.6 or above). After pH-induced flocculation, the magnesium content in the biomass was around 5 %. Overdosing of base during flocculation results in a slight increase of magnesium content in the biomass, but never excessively because of the low magnesium content of the water. Because the magnesium concentration in freshwaters is low, a large proportion of the magnesium disappears from solution during flocculation, about 0.25 – 0.5 mM magnesium. This depletion of magnesium from solution may pose problems when the culture medium is recycled, as the magnesium concentration in the recycled medium may be too low to induce

flocculation. Recycling of the culture medium is essential to minimize the need for water during production of microalgae biomass [34]. It is therefore important to recycle the magnesium that is consumed during flocculation. When magnesium is dissolved during the de-flocculation, it can be separated from the pre-concentrated biomass and returned to the culture medium to be used in a second round of flocculation. In our experiment, more than 95 % of the magnesium could be recycled.

In seawater, magnesium is the second most abundant cation after sodium. Because the magnesium concentration is very high in seawater, flocculation starts at a lower pH when compared to freshwater medium [17, 28]. Some precipitation of magnesium hydroxide and even some flocculation may already occur spontaneously due to photosynthetic rise of pH in the culture [35]. Overdosing of base can cause massive precipitation of magnesium and results in a large sludge volume and a very high magnesium content of the harvested biomass and this should be avoided [28]. The magnesium hydroxide that has precipitated can be easily removed from the biomass by mild acidification during the de-flocculation stage. The de-flocculation step has an additional advantage in that the calcium content of the harvested biomass is reduced. Precipitation of magnesium during flocculation barely lowers the magnesium concentration in the medium. Therefore, it is not essential to return the magnesium that is dissolved during the de-flocculation step to the culture.

In this study, flocculation was induced by addition of sodium hydroxide and de-flocculation was demonstrated using hydrochloric acid. In the most optimal scenario, flocculation of microalgae (0.5 g L^{-1}) was achieved after addition of 1.5 mM sodium hydroxide. This corresponds with a dose of 0.12 ton sodium hydroxide ton^{-1} microalgal biomass, resulting in a cost of 42 \$ ton^{-1} biomass (Table 3). However, this cost can be significantly reduced to 17 \$

415 ton^{-1} biomass if calcium hydroxide (slaked lime) is used instead of sodium hydroxide [15].
416 Based on our results, only 0.05 ton hydrochloric acid ton^{-1} biomass is needed for de-
417 flocculation. However, this would increase the cost of harvesting by at least 40 \$ ton^{-1}
418 biomass. The use of nitric acid could be an interesting alternative since the nitrate in nitric
419 acid could be used as nitrogen source for the microalgae when the medium is recycled. The
420 dosage of nitric acid that would be required for de-flocculation (0.75- 2.5 mM) corresponds
421 with the recommended nitrogen content of the microalgae cultivation medium (Table 1).

422 **Conclusion**

423
424 Our results show that magnesium can be effectively used as a pH-dependent reversible
425 flocculant for harvesting microalgae. At high pH, precipitation of magnesium hydroxide
426 results in flocculation of microalgae. At low pH, the magnesium is dissolved and removed
427 from the microalgae biomass. Thus, contamination of the harvested microalgae biomass by
428 the flocculant is avoided. The pH-shift required for flocculation and de-flocculation does not
429 affect the viability of the microalgae, nor the biochemical composition of the biomass. In
430 freshwater medium, the de-flocculation stage allows recycling of the magnesium and thus
431 avoids depletion of magnesium from the culture medium.
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440

Figures

Fig 1: Flocculation efficiency, pH after sodium hydroxide dosage and magnesium concentration in supernatant (S) and biomass pellet (P) for *Chlorella vulgaris* (A,B,C) and *Phaeodactylum tricornutum* (D,E,F)

Fig 2: Magnesium concentration in supernatant and biomass pellet before flocculation (BF), after pH-induced flocculation (AF) and after 30 and 60 min of de-flocculation at pH 6, 7 and 8 for (A) *Chlorella vulgaris* and (B) *Phaeodactylum tricornutum*.

Fig 3 : Comparison of growth using flocculated – de-flocculated biomass compared to a control inoculum for (A) *Chlorella vulgaris* and (B) *Phaeodactylum tricornutum*

Fig 4: Quantum Yield of PSII for *Chlorella vulgaris* and *Phaeodactylum tricornutum* (*Ptri*) before flocculation (BF), after flocculation (AF), after de-flocculation (60 min at pH 8) and a negative control of microalgae with 15% H_2O_2

Fig 5: Evan's Blue staining essay: *Chlorella vulgaris* (A) no treatment, (B) H_2O_2 for 30 min and (C) after de-flocculation 60 min pH 8 and *Phaeodactylum tricornutum*: (D) no treatment, (E) H_2O_2 for 30 min and (F) after de-flocculation 60 min pH 8

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559

Figure 1

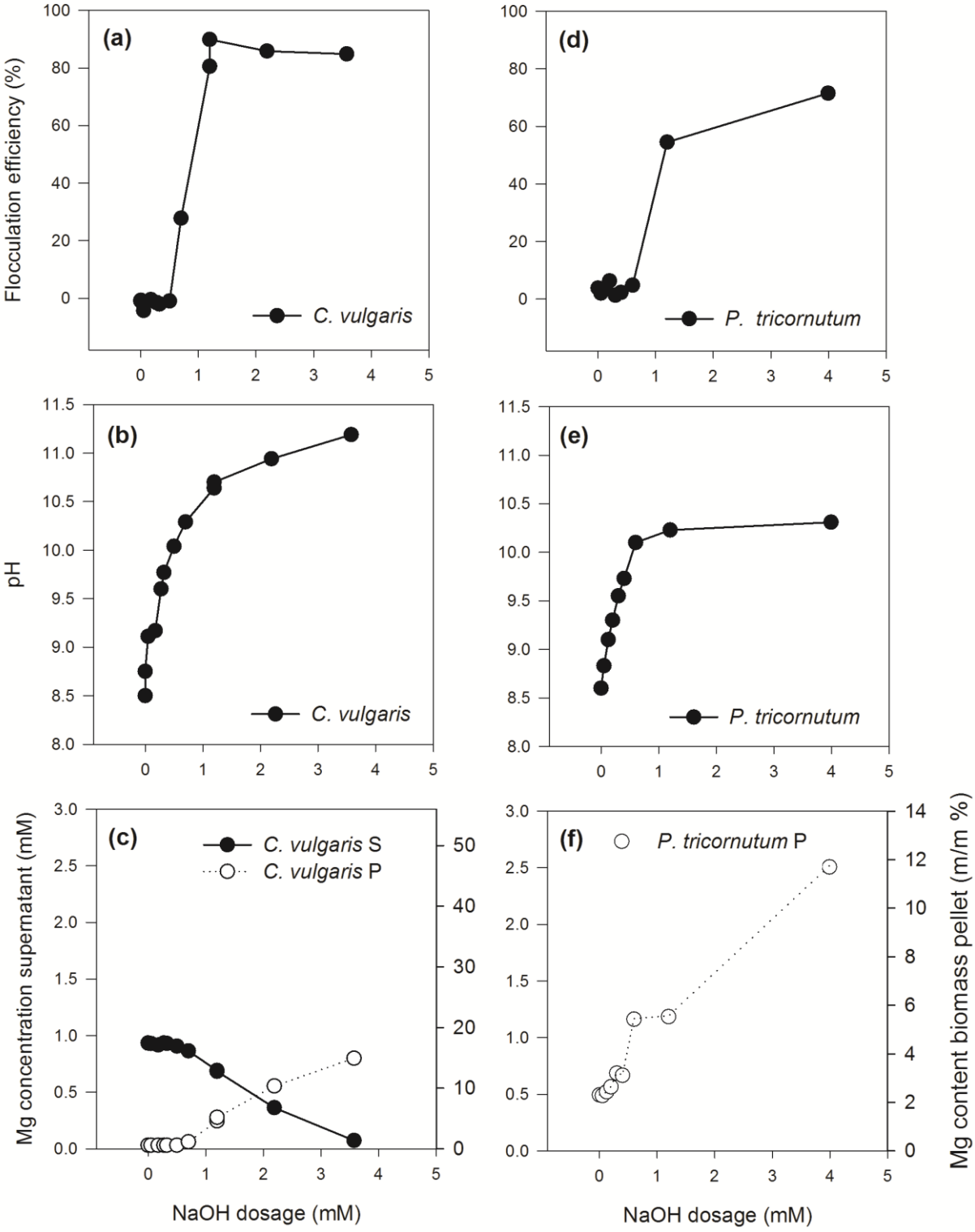

(a)
Flocculation efficiency (%)
C. vulgaris
(d)
P. tricornutum
(b)
pH
C. vulgaris
(e)
P. tricornutum
(c)
C. vulgaris S
C. vulgaris P
Mg concentration supernatant (mM)
NaOH dosage (mM)
(f)
P. tricornutum P
Mg content biomass pellet (m/m %)
NaOH dosage (mM)

Figure 2

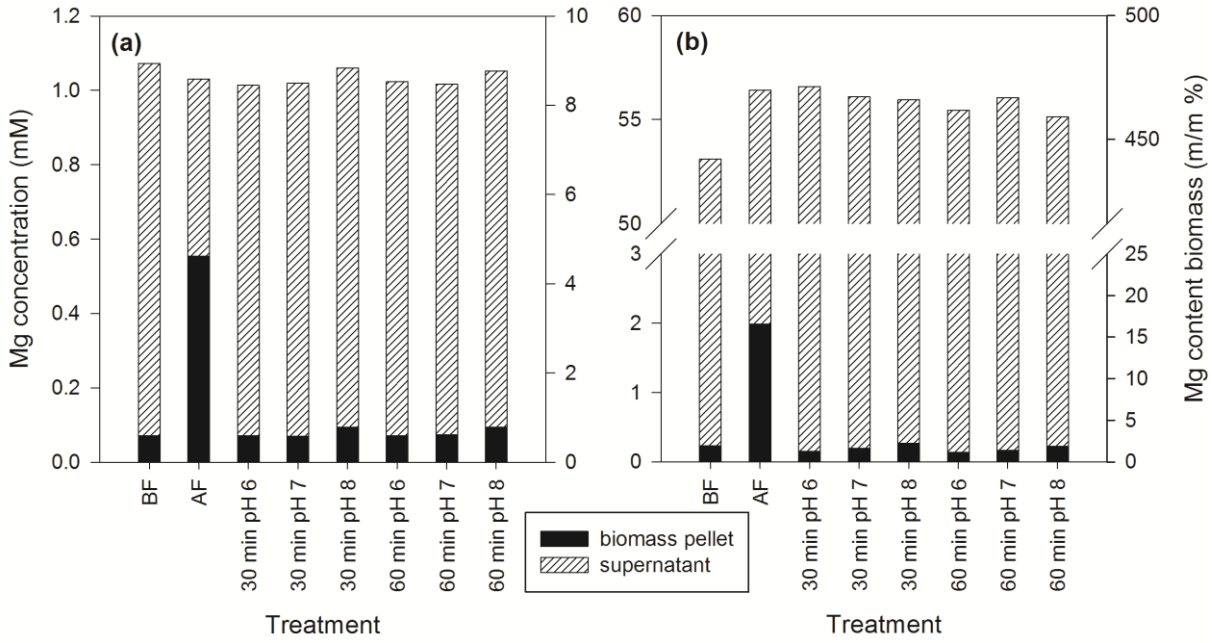


Figure 3

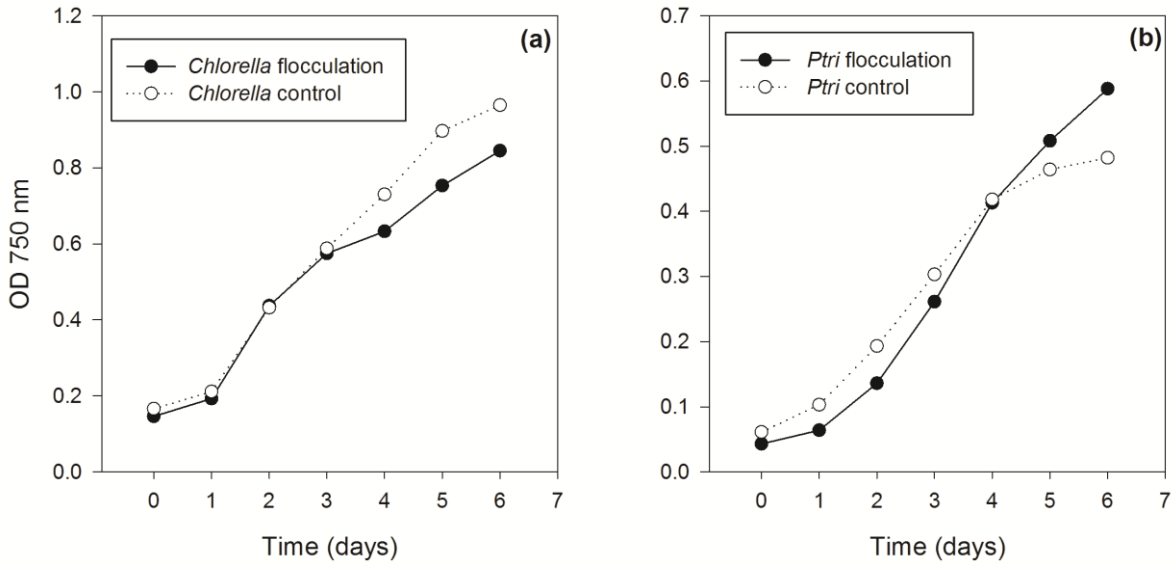
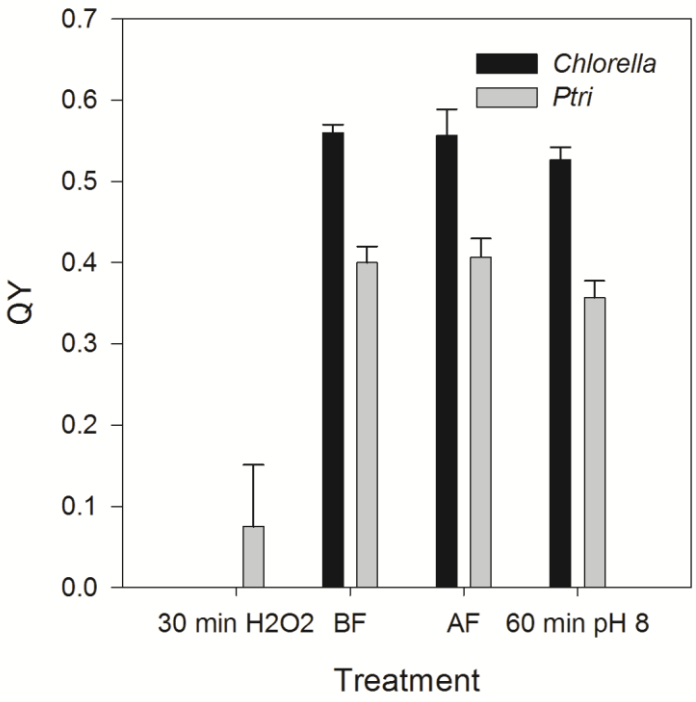
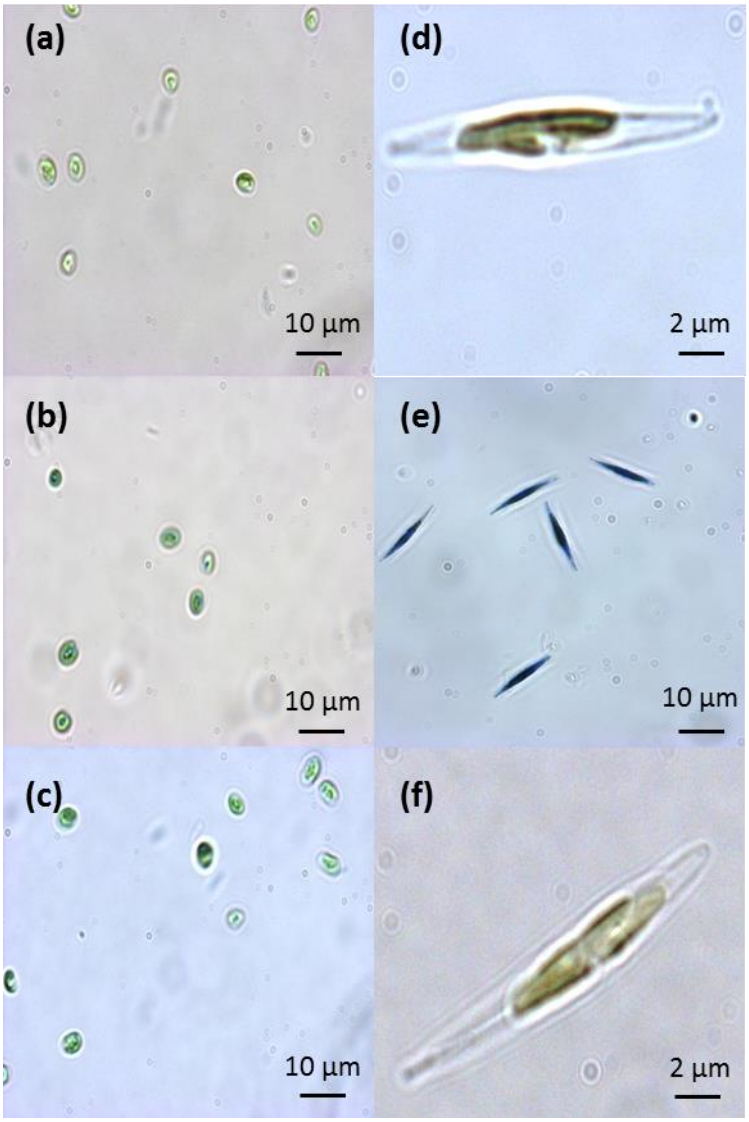


Figure 4


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
QY
Chlorella
Ptri
30 min H2O2
BF
AF
60 min pH 8
Treatment

571 **Figure 5**



572

573

Tables

Table 1. Concentrations of the main ions in the cultivation medium for *Chlorella vulgaris* (freshwater) and *Phaeodactylum tricornutum* (seawater) in batch cultivation mode

	C. vulgaris (mM)	*P. Tricornutum* (mM)
Cl^-	1.7	633.3
Na^+	1.9	569.3
Mg^{2+}	1.0	50.15
Ca^{2+}	2.7	10
K^+	0.3	0.3
NO_3^-	1	1
PO_4^{3-}	0.05	0.05
SO_4^{2-}	1.3	25

Table 2: Comparison of biomass composition in terms of carbohydrates, total FAME, proteins, Ca, Mg and P (m/m % dry weight) before (C) and after flocculation – de-flocculation (FDF) for *Chlorella vulgaris* and *Phaeodactylum tricornutum*

content (%)	*Chlorella vulgaris*			*Phaeodactylum tricornutum*		
	C	FDF	P-value	C	FDF	P-value
Carbohydrates	31.2	27.9 ± 1.85	0.489	13.9	14.5 ± 1.09	0.445
FAME	24.3	25.8 ± 1.16	0.155	12.1	13.6 ± 0.69	0.066
Proteins	22.4	22.9 ± 0.57	0.284	27.8	28.8 ± 1.75	0.404
Ca	0.22	0.11 ± 0.02	0.008	0.55	0.32 ± 0.13	0.093
Mg	0.42	0.57 ± 0.12	0.162	0.87	0.77 ± 0.17	0.407
P	0.57	0.51 ± 0.08	0.324	0.24	0.24 ± 0.01	0.240

Table 3: Cost calculation based on optimal scenario for reversible magnesium flocculation

Flocculation	
Biomass concentration (g L^{-1})	0.5
Dose NaOH (ton ton^{-1} biomass)	0.12
[1]Cost NaOH ($ ton^{-1} biomass)	42
Dose $Ca(OH)_2$ (ton ton^{-1} biomass)	0.11
[2]Cost $Ca(OH)_2$ ($ ton^{-1} biomass)	17
De-flocculation	
Dose HCl (ton ton^{-1} biomass)	0.05
[3]Cost HCl ($ ton^{-1} biomass)	40

[1] NaOH industrial grade \$350 ton^{-1}

[2] $Ca(OH)_2$ slaked lime \$150 ton^{-1}

[3] HCl industrial grade 35% \$250 ton^{-1}