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Influence of organic matter on flocculation of *Chlorella vulgaris* by calcium phosphate precipitation

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Abstract

Flocculation is a promising approach for reducing the cost of harvesting microalgae. Flocculation of microalgae can be induced by precipitation of calcium phosphate (Ca-phosphate) when pH increases above 8.5, a pH level that can be achieved by simple photosynthetic CO_2 depletion. Using the freshwater microalgae *Chlorella vulgaris* as a model, we identified the combinations of minimum pH and Ca and PO_4 concentrations to induce flocculation. Predicted concentrations of amorphous $Ca_3(PO_4)_2$ precipitation (chemical modelling) explained flocculation in these solutions. The efficiency of flocculation decreased with increasing microalgal biomass concentration. Solution renewal experiments suggest that flocculation is inhibited by algal organic matter in the medium, even when present at relatively low concentrations relative to concentrations in stationary phase medium. Addition of dissolved organic compounds showed that organic acids with a high molecular weight (e.g. humic acids, alginate) have a strong inhibitory effect on flocculation whereas glucose or acetate had no such effect. These effects may be related to complexation of Ca^{2+} or effects of organic matter on growth of the Ca-phosphate crystals. Precipitation of Ca-phosphate in media with high organic matter content requires a high water hardness (500 µmol L^{-1} Ca) and high PO_4 concentrations (350 µmol L^{-1} P). Flocculation can be facilitated by addition of surplus PO_4 to the medium. This surplus PO_4 may be recovered after flocculation by re-dissolution of the Ca-phosphate through mild acidification.

1. Introduction

Microalgae have a higher areal productivity than agricultural crops while using less land and water, therefore they are a promising novel source of biomass for production of biofuels [1]. At present, however, the costs and energy requirements for algal biomass production are too high for microalgal biofuels to compete with first generation biofuels [2]. Harvesting is a major bottleneck in cost-efficient production of microalgae, contributing 20 - 30 % to the total production cost [3,4] and is an important hurdle to take to realize microalgal biofuels.

Algal harvesting is difficult because of the small size of algal cells (3 – 30 µm) and the low biomass concentration (0.2 - 0.6 g L^{-1}). Flocculation is a promising approach to reduce the cost of harvesting microalgae, as it allows separation of microalgae from the medium by simple gravity sedimentation [5]. Flocculation can be induced by metal coagulants such as alum or ferric chloride or by polymeric flocculants such as polyacrylamides or chitosan. However, this requires addition of chemicals and thus results in contamination of either the microalgal biomass, the treated water or both. Flocculation of microalgae can also occur spontaneously without the need for chemicals, a phenomenon that is referred to as autoflocculation or bioflocculation. Auto- and bioflocculation are considered as promising approaches for harvesting microalgal biomass [6,7]. Bioflocculation describes flocculation caused by biopolymers produced by algae or by bacteria. Autoflocculation is the phenomenon where microalgae flocculate as a result of the pH increase of the medium due to photosynthetic consumption of carbon dioxide [8]. This flocculation is the result of precipitation of Ca-phosphates. Ca-phosphate flocculation is a particularly attractive option when wastewater is used as a source of nutrients for production of biofuels, since wastewater often contains ample PO_4. Recent studies indicate that combination of microalgal biofuel production with wastewater treatment offers a sustainable and economically attractive approach to production of microalgae, as there is no need for synthetic fertilizers and additional income can be generated through treatment of wastewater [9].

Sukenik and Shelef [10] investigated the underlying mechanism of flocculation of microalgae by Ca-phosphate precipitates. They noted that flocculation can be induced within a pH range of 8.5 - 9 if the culture medium contains sufficient amounts of Ca (1500 - 2500 µmol L^{-1}) and PO_4 (100 - 200 µmol L^{-1}). They also demonstrated that Ca and PO_4 precipitate during flocculation and that these precipitates are involved in the flocculation of the algal cells [11]. Sukenik et al. [10,11] studied Ca-phosphate flocculation under laboratory conditions. In real systems, however, this flocculation is often unpredictable and the flocculation efficiency tends to be low, even when conditions appear to be favorable [12].

It is not clear why flocculation by Ca-phosphate precipitates fails even though Ca and PO_4 concentrations and pH are sufficiently high for the mechanism to occur. A possible explanation may be the interference with dissolved organic matter (DOM) in the medium. The DOM may be present in the wastewater (e.g. humic substances) or be produced as extracellular organic matter by the microalgae (algal organic matter or AOM). It is known that several organic compounds may interfere with Ca-phosphate precipitates by complexation of Ca^{2+} or by reducing crystal growth, thus preventing the formation of the Ca-phosphate precipitates required to induce flocculation [13–15].

Alternatively, organic matter present in the medium may also compete with microalgal cells for positive charges of the flocculant and thus increase the required flocculant dose [16–18]. A better understanding of the influence of organic matter on flocculation by Ca-phosphate precipitates may lead to a more reliable use of this potentially cost-efficient harvesting method.

Despite the fact that autoflocculation by Ca-phosphate precipitation was considered a promising harvesting method in the 1980's, this flocculation method has not received much attention in recent years. The objective of this study was, therefore to re-evaluate the potential of this flocculation method for harvesting microalgae. In particular, we aimed at investigating how DOM or AOM present in the culture medium may interfere with flocculation by Ca-phosphate precipitates. We use *Chlorella vulgaris* as a model species as it is often used for microalgal flocculation and it is generally dominant in microalgal wastewater treatment systems.

2. Materials and methods

2.1 Algal cultivation

Chlorella vulgaris (strain 211-11b, SAG, Germany) was used as a model species. *Chlorella* is a common species in pilot systems for wastewater treatment using algae and is also a promising species for biofuel production [19–21]. *Chlorella* was cultured in Wright's Cryptophyte (WC) medium [22]. Ca and PO_4 concentrations in the medium (250 µmol L^{-1} Ca and 50 µmol L^{-1} PO_4) are relatively low compared to the concentrations required to induce flocculation. *Chlorella* was cultured in a 30 L plexiglass bubble column photobioreactor (20 cm diameter) stirred by aeration with 0.2 µm filtered air (5 L min^{-1}). The pH of the culture medium was controlled at pH 8 through addition of carbon dioxide to the air flow. The culture was irradiated from two sides with daylight fluorescent tubes, giving a light intensity of 60 µE m^{-2} s^{-1} at the surface of the reactor. Algal biomass was monitored by measuring absorbance at 750 nm [23]. Absorbance measurements were converted to dry weight biomass by calibration against gravimetrical measurements after filtration of a subsample on preweighed GF/F glass fibre filters. Experiments were carried out when the culture was in early stationary phase (1 week old) and when biomass concentrations were 0.2-0.3 g L^{-1}. As the WC medium used is phosphorus-limited, PO_4 concentrations at this stage were low.

2.2 Flocculation experiments

Jar test experiments were used to quantify the efficiency of *Chlorella* flocculation in different media and at different pH levels. All experiments were carried out in triplicate. *Chlorella* suspensions were transferred to 100 mL beakers that were stirred by a magnetic stirrer. pH was adjusted by addition of 0.5 mol L^{-1} HCl or 0.5 mol L^{-1} NaOH. The microalgal suspension was mixed intensively (105 rad s^{-1}) for 10 min during and just after pH adjustment. Then, the suspensions were allowed to settle for 30 min. Optical density at 750 nm was measured prior to pH adjustment (OD_i) and after settling (OD_f). The flocculation efficiency (η_a), or the percentage of algal biomass removed from suspension, was calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} . 100 \quad (1)$$

A flocculation efficiency higher than 80% was considered to be effective flocculation.

2.3 Flocculation in the absence of organic matter

A series of experiments were carried out to determine minimum pH and Ca and PO_4 concentrations required to induce flocculation under optimal conditions, i.e. in the absence of algal organic matter or AOM in the medium. The AOM was removed by separating the microalgae from the original medium using centrifugation and by resuspending them in an equal volume of fresh medium prior to the experiments. Preliminary experiments had shown that centrifugation and subsequent resuspension in the same medium had no significant effect on flocculation of *Chlorella* cells but resulted in a reduction in polysaccharide concentrations in the medium by an order of magnitude [18]. After resuspension in fresh medium, pH of the microalgal suspension was adjusted to 7. The Ca and PO_4 concentrations were adjusted by addition of a 1000 mmol L^{-1} Ca solution and a 100 mmol L^{-1} PO_4 solution. All possible combinations of four Ca and four PO_4 concentrations were prepared (250, 500, 850 and 1800 µmol L^{-1} Ca and 50, 150, 350 and 1000 µmol L^{-1} PO_4). The lowest concentration of Ca and PO_4 corresponded with the concentrations in the WC medium. The highest Ca concentration tested was typical of hard water. The pH was increased stepwise in 0.5 pH unit intervals up to pH 10 by addition of 0.5 mol L^{-1} NaOH. The upper limit of pH 10 was chosen as this is the maximum pH that could be achieved due to photosynthesic CO_2 depletion in our *Chlorella* cultures, which is similar to what has previously been observed by others [10,24]. Limiting the pH increase to 10 also prevented flocculation due to precipitation of magnesium hydroxide, which occurs only above pH 10.5 [25]. The flocculation efficiency η_a was measured after each stepwise pH increase.

According to Sukenik et al [11] amorphous Ca-phosphate precipitates are formed during pH increase in algal suspensions with sufficient Ca and PO_4. Therefore we calculated the concentration of amorphous $Ca_3(PO_4)_2$ formed, using Visual Minteq 3.0. The amorphous form has a log K_{sp} of -25.5 and we entered the nominal medium composition and a temperature of 25 °C. The calculated precipitate concentrations for the different treatments were compared to the measured flocculation efficiency of each treatment.

Additional experiments were carried out to verify the underlying mechanism of flocculation in our experimental setup. To verify that Ca-phosphate precipitation indeed occurred during flocculation, total Ca and PO_4 concentrations were measured in the liquid and the pellet phase using ICP-OES (Perkin Elmer, Optima 3300 DV) before and after flocculation. To evaluate whether flocculation was due to charge neutralisation, we determined the zeta potential of both the Ca-phosphate precipitates formed at high pH in deionised water and of the *Chlorella* cells during flocculation (using a Malvern Zetasizer Nano) [26]. Zeta potential measurements were carried out during pH increase in a medium with 1800 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4. If flocculation occurs by charge neutralisation, the flocculation efficiency is expected to decrease with increasing microalgal biomass concentrations. To confirm this, we determined the flocculation efficiency η_a at different pH levels of *Chlorella* suspensions with differing biomass concentrations but with identical Ca and PO_4 concentrations and pH.

2.4 Influence of organic matter on flocculation

To evaluate the influence of AOM excreted by *Chlorella* in the culture medium, we compared flocculation between *Chlorella* cells resuspended in fresh medium without AOM and that of cells resuspended in their original medium containing AOM. These solution renewal experiments were carried out at the same Ca and PO_4 concentrations as in the absence of AOM, using the same experimental setup. As these experiments demonstrated a significant inhibition of flocculation in original medium, we aimed to investigate the relative concentration of AOM required to inhibit flocculation. Therefore, *Chlorella* cells were resuspended in its original medium diluted with various proportions of fresh medium lacking AOM (0, 25, 50, 75 and 100 % original medium). Ca (500 µmol L^{-1}) and PO_4 (350 µmol L^{-1}) were added, pH was adjusted to 9.5 and the flocculation efficiency η_a was measured.

Finally, we tested which specific classes of organic compounds interfered with flocculation. Therefore, we investigated inhibition of flocculation of *Chlorella* cells resuspended in fresh medium containing 850 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4 at pH 9.5. We tested inhibition by the organic acids citric acid (20 and 50 mg L^{-1}) and Ca-acetate (20 and 50 mg L^{-1}), as organic acids are known to interfere with Ca-phosphate crystallisation [13]. We also tested glucose (20 and 50 mg L^{-1}) and Na-alginate (20 and 50 mg L^{-1}) as a model for carbohydrates excreted by algae in their medium [27]. Finally, we also tested humic substances (Aldrich humic acid, 20 and 200 mg L^{-1}) as these may be present in the medium if wastewater is used as a source of nutrients for algal biomass production.

2.5 Recovery of phosphate after flocculation

As flocculation of microalgae by precipitation of Ca-phosphate requires high PO_4 concentrations in the medium when AOM is present, we explored the possibility to recover the PO_4 from the microalgae after flocculation. We first induced flocculation by Ca-phosphate precipitation at pH 8 in medium containing 1800 µmol L^{-1} Ca and 1000 µmol L^{-1} PO_4. We then reduced the pH to 6 and stirred the solution for 30 minutes. The PO_4 concentrations in the medium were compared before flocculation, after flocculation and after pH-reduction for dissolution of Ca-phosphates. During these treatment steps the quantum yield of photosystem II was also measured to evaluate the stress experienced by the microalgal cells during the pH-fluctuations (using a PSI AquaPEN PAM fluorometer).

3. Results and discussion

3.1 Flocculation under optimal conditions

The influence of Ca and PO_4 concentrations and pH on flocculation of *Chlorella* cells was investigated in fresh medium lacking AOM (Figure 1). These experiments showed that flocculation was in general favoured by high Ca and high PO_4 concentrations and that a higher pH was required to induce flocculation when Ca and PO_4 concentrations were low. No flocculation occurred within the studied pH range when PO_4 concentrations were only 50 µmol L^{-1}. When PO_4 concentrations were 150 µmol L^{-1}, flocculation occurred when Ca concentrations were 850 µmol L^{-1} or higher, which corresponds to Ca concentrations in moderately hard to hard water. When PO_4 concentrations were 350 µmol L^{-1} or higher, flocculation could also be induced at low Ca concentrations, but lower Ca concentrations required a higher pH for flocculation to occur. These results agree well with previous studies that carried out similar experiments [11,28].

We assumed that flocculation was the result of precipitation of Ca-phosphates at high pH (as suggested by [11]). This was supported by measurements of Ca and PO_4 by ICP, which showed that flocculation of *Chlorella* cells in our experiments was associated with a shift of Ca and PO_4 from the liquid medium to the algal pellet. During flocculation, Ca concentration decreased from 741 ± 3 µmol L^{-1} to 432 ± 16 µmol L^{-1} and PO_4 concentration decreased from 244 ± 1 µmol L^{-1} to 56 ± 2 µmol L^{-1}, roughly confirming to a removal of ions at the 3:2 molar Ca:P stoichiometry of $Ca_3(PO_4)_2$. The observed decrease by 42% for Ca and 77% for PO_4 corresponded well with the decrease predicted by Visual Minteq modelling of amorphous $Ca_3(PO4)_2$ precipitation of 42% for Ca and 68% for P (details not shown).

If flocculation was indeed induced by precipitated Ca-phosphates, the flocculation efficiency would be expected to be related to the quantity of Ca-phosphate precipitates formed. The measured flocculation efficiency was clearly dependent on the quantity of precipitated amorphous Ca-phosphates, estimated using Visual Minteq modelling (Figure 2). No flocculation occurred below 30 µmol L^{-1} of Ca-phosphate precipitates formed and always occurred above 100 µmol L^{-1} Ca-phosphate precipitate. Between 30 and 100 µmol L^{-1} Ca-phosphate precipitate, flocculation was variable. These observations are in agreement with our assumption that a certain quantity of precipitate is needed to induce flocculation of the algal cells.

The Ca-phosphate precipitates may cause flocculation by sweeping flocculation or charge neutralisation [29]. If charge neutralisation is the dominant mechanism, the Ca-phosphate precipitates should carry a positive charge in order to be capable of neutralising the negative surface charge of the microalgal cells. Zeta potential measurements indeed showed that particles with a positive charge of about 10 mV were formed in a Ca and PO_4 solution above pH 8.5. Measurements of the zeta potential in a suspension of *Chlorella* cells increased from negative values to neutral values when flocculation occurred (Figure 3). This suggests that charge neutralisation may be responsible for or at least contribute to flocculation.

If flocculation is caused by charge neutralisation, theoretically, a higher amount of Ca-phosphate precipitates would be required to induce flocculation if the biomass concentration increases. Thus, increasing the biomass of microalgae while keeping Ca and PO_4 concentrations constant should result in an increase in the pH required to induce flocculation and ultimately a decrease of the flocculation efficiency. Indeed, when different biomass concentrations of *Chlorella* cells were added to a medium with the same Ca and PO_4 concentrations, flocculation occurred at a pH of 8.5 when the biomass concentration was only 50 mg L^{-1} and the pH increased to 9.5 when the biomass concentration was 250 mg L^{-1} (Figure 4). When the biomass concentration was 500 mg L^{-1} or higher, flocculation could no longer be induced. This suggests that indeed more Ca-phosphate precipitate is needed to induce flocculation when microalgal biomass concentration is higher. In a previous study of flocculation of microalgae by Ca-phosphate precipitation, Sukenik et al. [11] also provided evidence that flocculation is caused by charge neutralisation.

It should be noted, however, that the surface charge of Ca-phosphates depends on the residual concentrations of Ca and PO_4 in the medium, the pH and sorption of other ions such as Mg onto the Ca-phosphate surface. A high ratio of residual Ca over PO_4 is required to induce a positive surface charge on Ca-phosphate precipitates [30]. In our experiment where the zeta potential was monitored as well as in the experiments by Sukenik et al. [11], the residual Ca to PO_4 ratio was higher than 1.5 and thus the surface charge of the precipitates would be positive. In other treatments (Figure 1), on the contrary, the residual Ca to PO_4 ratio was lower than 1.5. Yet this did not seem to inhibit flocculation. This suggests that other mechanisms such as sweeping flocculation may also be important. More detailed research is needed to elucidate which mechanisms are involved in flocculation by Ca-phosphates.

3.2 Influence of growth medium replacement

Microalgae excrete large amounts of AOM in their growth medium [31,32]. Up to 17 % of the organic matter in dense microalgal cultures may be present as dissolved organic matter [33]. To evaluate the influence of medium replacement on flocculation we investigated the flocculation of *Chlorella* cells resuspended in their original culture medium, containing AOM, and compared it with cells resuspended in fresh medium lacking AOM (Figure 1). In original medium, flocculation could not be induced over the pH range studied when PO_4 concentrations were 150 µmol L^{-1} or lower. When PO_4 concentrations were 350 µmol L^{-1}, flocculation could only be induced when Ca concentrations were 500 µmol L^{-1} or higher. In the treatments with high Ca and PO_4 concentrations where flocculation could be induced, flocculation occurred at a higher pH than in the experiments with medium replacement. This points to a strong inhibition of flocculation, likely caused by organic compounds present in the microalgal cultures. Recently, Vandamme et al. [18] also showed that AOM interferes with flocculation of microalgae using five different flocculation techniques (aluminum sulphate, electro-coagulation-flocculation, chitosan, cationic starch and pH induced flocculation).

We resuspended *Chlorella* cells in fresh medium mixed in various proportions with the original medium to study whether medium replacement has an inhibiting effect at lower concentrations than those present in the original stationary phase medium (Figure 5). While the flocculation efficiency η_a was > 90% in fresh medium, this decreased to 40%

when fresh medium was mixed with only 25% of the original medium. When the proportion of the original medium in the mixture was 50% or higher, flocculation was completely inhibited. This suggests that even lower concentrations of AOM than those present in the stationary phase medium can have an important inhibitory effect on flocculation caused by Ca-phosphate precipitates.

To explore which organic compounds may interfere with flocculation, we carried out a series of experiments in which flocculation of *Chlorella* cells was studied in medium to which specific organic compounds were added (Table 1). Addition of glucose and acetate to the fresh medium had no inhibitory effect on flocculation. Addition of low concentrations of citric acid (20 mg L^{-1}) and humic acids (20 mg L^{-1}) had no or only a weak effect on flocculation but high concentrations of citric acid (50 mg L^{-1}) or humic acid (200 mg L^{-1}) had a strong inhibitory effect. Addition of alginate completely inhibited flocculation both at low (20 mg L^{-1}) and high (50 mg L^{-1}) concentrations. Organic compounds may inhibit flocculation by preventing Ca-phosphate precipitation. Several studies have shown that specific organic compounds have an inhibitory effect on precipitation of Ca-phosphates. Precipitation may be inhibited through complexation of Ca^{2+}, thus reducing the effective concentration of Ca in the medium. Precipitation may also be inhibited by binding of organic compounds to the active growth sites on the surface of the Ca-phosphate crystals, thus preventing growth of the Ca-phosphate precipitates. Studies on the influence of organic ligands on precipitation of Ca-phosphates have shown that alginates, humic acids and citrate but not acetate and glucose inhibit precipitation of Ca-phosphates [13,14,34,35].

3.3 Practical implications

Flocculation by Ca-phosphate precipitates has the potential to be a cost-efficient method to harvest microalgae, as it allows flocculation of microalgae without the need for chemicals other than those present in the medium. Flocculation of microalgae can theoretically be induced within the pH range that typically occurs in algal production systems (pH 8-10) without the need for addition of alkalinity. Flocculation is dependent on the microalgal biomass concentration, with high biomass concentrations requiring higher Ca and PO_4 concentration and/or a higher pH to induce flocculation.

Although flocculation could theoretically occur at a low pH and relatively low Ca and PO_4 concentrations, our results clearly show that the presence of original growth medium inhibits flocculation and will result in higher Ca and PO_4 concentrations and or a higher pH to induce flocculation. This may explain why flocculation is often unreliable in real systems, e.g. in high rate algal ponds in which algae are used for wastewater treatment [36]. High-molecular weight organic acids like alginate-like polysaccharides excreted in the medium as AOM or humic acids present in the medium may have a particularly strong inhibitory effect. Even low concentrations of AOM appear to inhibit flocculation. Humic acids may pose a significant problem when agricultural waste streams such as liquid manure or effluents from biomass anaerobic digesters are used as a source of nutrients for microalgae production.

Because flocculation depends on the presence of relatively high concentrations of PO_4 in the medium, this harvesting method may be particularly attractive when microalgal biomass production is combined with wastewater treatment. Many wastewaters have a

N:P ratio < 16, which is below the ratio in which microalgae consume N and P [6]. Ca-phosphate precipitates can be used to remove surplus P from the wastewater that is not consumed by microalgae. When original growth medium, containing AOM, is present flocculation can only be used for harvesting microalgae if PO_4 concentrations exceed 350 µmol L^{-1} and Ca concentrations exceed 500 µmol L^{-1}. These conditions are typical for relatively high P loaded wastewaters and moderately hard to hard waters. This precludes the use of domestic wastewaters, which have PO_4 concentrations well below 300 µmol L^{-1} in regions where PO_4-based detergents have been banned.

Adding surplus PO_4 to the culture medium only to allow flocculation by Ca-phosphate precipitation to occur is not cost-efficient, given the high cost of synthetic phosphates. It is also not sustainable due to declining phosphate reserves [37]. Addition of surplus PO_4 to the medium, however, may be warranted if the PO_4 can be easily recovered from the harvested microalgal biomass after flocculation. We tested this possibility by attempting to re-dissolve the precipitated PO_4 after flocculation by acidifying the medium. During flocculation at a pH of 8, dissolved PO_4 concentration in the medium decreased from 800 ± 20 µmol L^{-1} to 670 ± 60 µmol L^{-1}. After reducing the pH to 6 for half an hour, PO_4 concentration was returned to 820 ± 20 µmol L^{-1}, indicating that all the precipitated PO_4 could be returned to solution by mild acidification. The fluctuations in pH (from 7 to 8 and back to 6) during flocculation and re-dissolution of PO_4 did not appear to affect the viability of the *Chlorella* cells as the quantum yield of photosystem II remained constant (0.647 ± 0.013 before flocculation and 0.635 ± 0.006 after re-dissolution of PO_4). This suggests that the PO_4 can indeed be recovered after flocculation without a major impact on the viability of the microalgae. Re-dissolution of the PO_4 after flocculation has the additional advantage that the biomass is not contaminated with Ca-phosphate precipitates as a result of the harvesting process.

4. Conclusions

Flocculation of *Chlorell*a by Ca-phosphate precipitates can be induced at a relatively low pH if Ca and PO_4 concentrations are sufficiently high. Such a pH can theoretically be achieved simply by photosynthetic depletion of CO_2 from the culture medium and therefore does not require addition of base. Dissolved organic matter, however, interferes with this process, resulting in higher Ca and/or PO_4 concentrations and/or a higher pH to induce flocculation. This is probably an important reason why flocculation by this method is unpredictable in microalgal cultures. Organic acids with a high molecular weight such as humic acids or alginate-like substances have a strong inhibitory effect on flocculation, probably because they prevent precipitation of Ca-phosphate. In practice, the use of flocculation by Ca-phosphate precipitates is a feasible option only when relatively concentrated wastewaters are used as a source of nutrients and when water hardness is sufficiently high. Alternatively, extra PO_4 can be added to the culture medium to induce flocculation. This PO_4 may subsequently be recovered by re-dissolution by reduction of the pH after flocculation.

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Fig. 1. Flocculation efficiency of *Chlorella vulgaris* as a function of pH in media with different concentrations of Ca and PO_4. To assess the influence of algal organic matter (AOM) on flocculation, the flocculation efficiency was assessed in fresh medium without AOM (AOM-) and in the original medium with AOM (AOM+).

Fig. 2. The flocculation efficiency of *Chlorella vulgaris* as a function of the amount of precipitated amorphous $Ca_3(PO_4)_2$. Flocculation efficiencies were measured under the experimental conditions (pH, Ca and PO_4 concentration) shown in Fig. 1 (AOM-) while the concentration of precipitated amorphous $Ca_3(PO_4)_2$ was modelled for the specific conditions using Visual Minteq.

Fig. 3. Zeta potential of *Chlorella vulgaris* suspension in fresh medium containing 1800 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4. Flocculation under these conditions occurred at pH 8.5.

Fig. 4. The influence of biomass concentration of *Chlorella vulgaris* on the flocculation efficiency at different pH levels. For these experiments, different quantities of *Chlorella vulgaris* cells were resuspended in fresh medium containing 850 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4 to achieve different biomass concentrations.

Fig. 5. The influence of different proportions of original medium containing AOM mixed with fresh medium without AOM on the flocculation efficiency of *Chlorella vulgaris*. The flocculation efficiency was determined at pH 9.5 in a medium containing 500 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4.

Table 1. Comparison of the flocculation efficiency of Chlorella vulgaris cells in fresh medium without AOM, in fresh medium without AOM to which different model organic compounds were added, and in the original medium containing AOM. The flocculation efficiency was determined at pH 9.5 in fresh medium containing 850 µmol L^{-1} Ca and 350 µmol L^{-1} PO_4.

Organic compounds	**Flocculation efficiency (%)**
Fresh medium (AOM-)	85
Glucose 20 mg/L	97
Glucose 50 mg/L	95
Acetate 20 mg/L	97
Actetate 50 mg/L	95
Alginate 20 mg/L	0
Alginate 50 mg/L	1
Humic acid 20 mg/L	98
Humic acid 200 mg/L	38
Citric acid 20 mg/L	70
Citric acid 50 mg/L	0
Original medium (AOM+)	0

Table 1

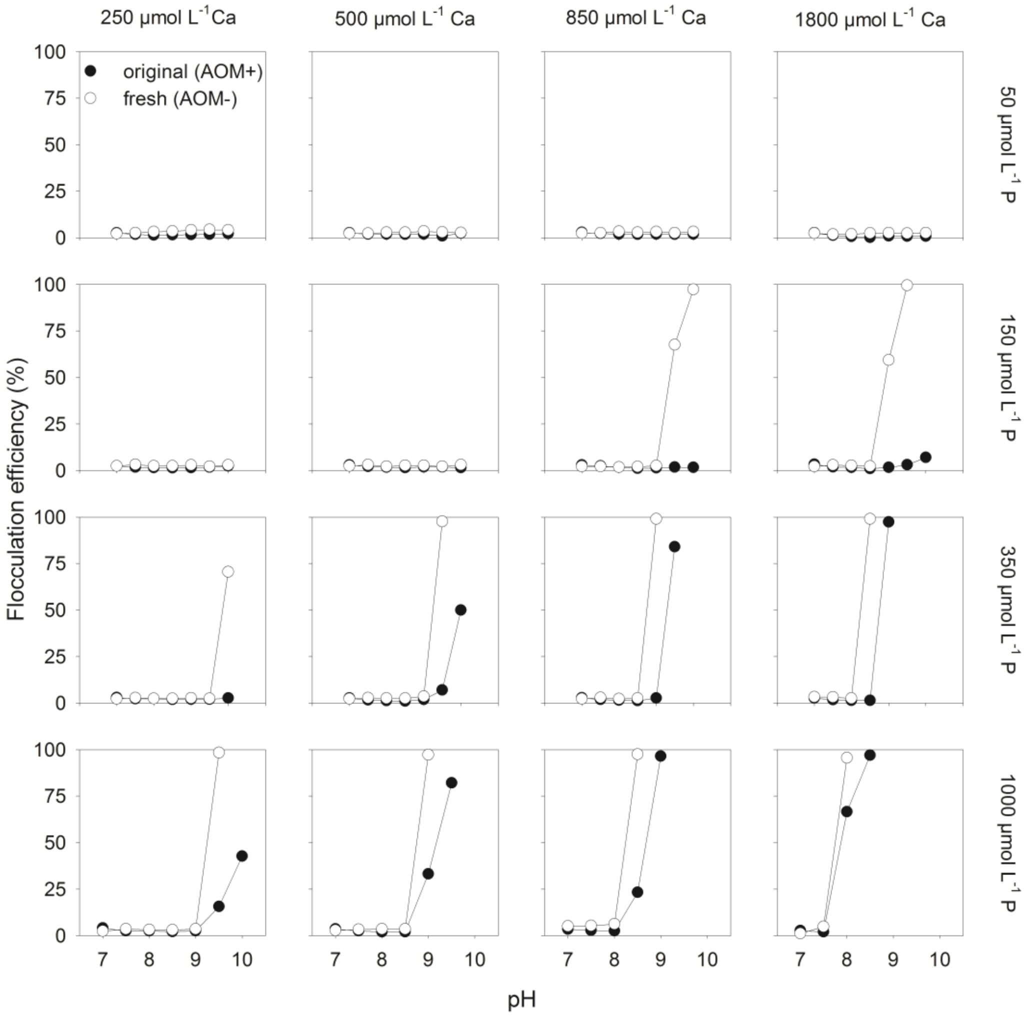
250 µmol L⁻¹Ca
500 µmol L⁻¹ Ca
850 µmol L⁻¹ Ca
1800 µmol L⁻¹ Ca
original (AOM+)
fresh (AOM-)
50 µmol L⁻¹ P
150 µmol L⁻¹ P
350 µmol L⁻¹ P
1000 µmol L⁻¹ P
Flocculation efficiency (%)
0
25
50
75
100
7
8
9
10
pH

Figure 1

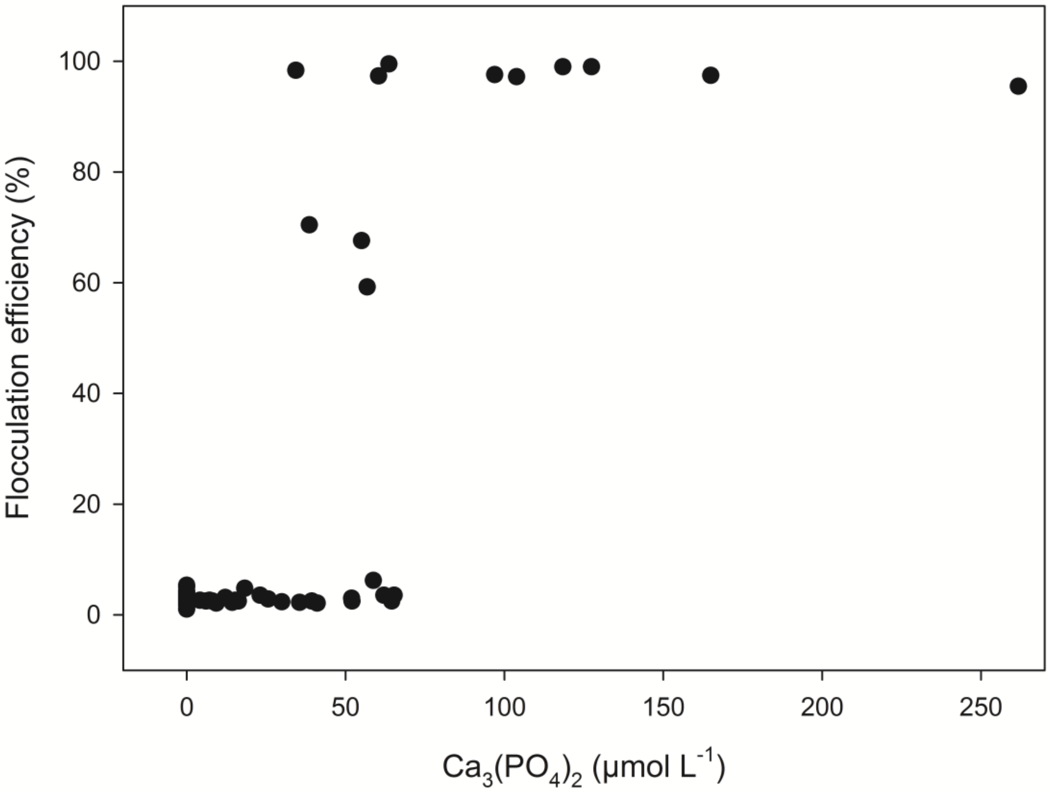
Flocculation efficiency (%)
100
80
60
40
20
0
0
50
100
150
200
250
$Ca_3(PO_4)_2$ (µmol L^{-1})

Figure 2

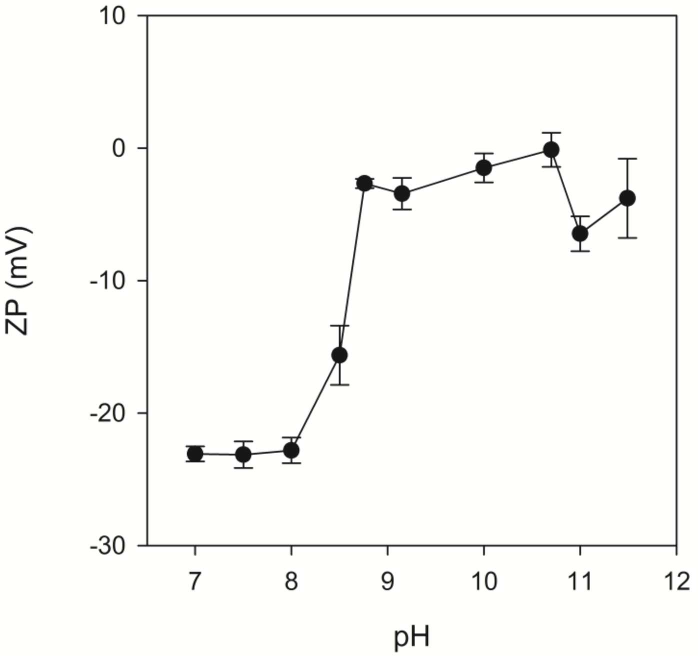

Figure 3

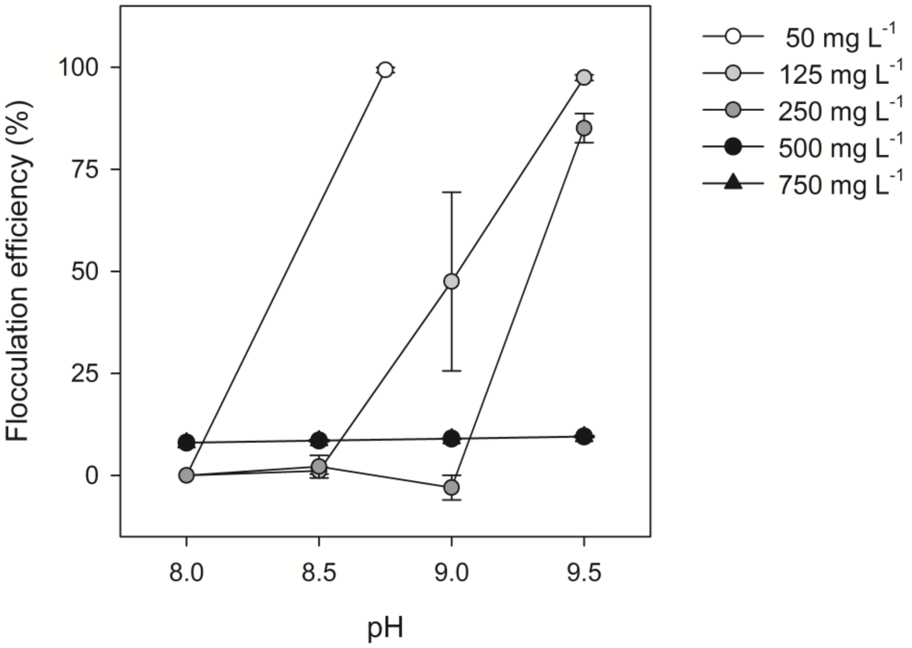
Flocculation efficiency (%)
100
75
50
25
0
8.0
8.5
9.0
9.5
pH
50 mg L-1
125 mg L-1
250 mg L-1
500 mg L-1
750 mg L-1

Figure 4

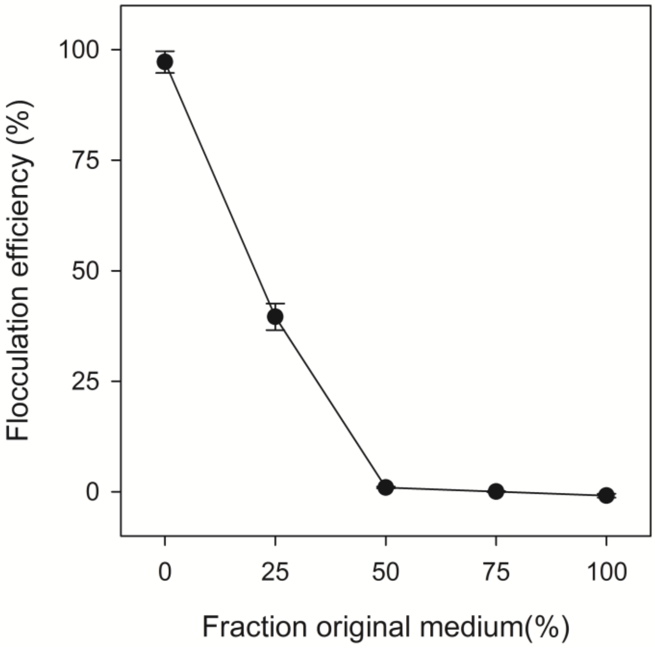
Flocculation efficiency (%)
100
75
50
25
0
0
25
50
75
100
Fraction original medium(%)

Figure 5