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Effect of hydroxyapatite, octacalcium phosphate and calcium phosphate on the auto-flocculation of the microalgae in a high-rate algal pond

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Recovering microalgae is one of the main technological and economic concerns in a high-rate algal pond (HRAP) because of their small size and their low density. This paper emphasizes the characterization (identification and assessment of potential flocculation) of chemical compounds involved in microalgae auto-flocculation in a HRAP. First, thermodynamic simulations were performed, using two models (i.e. Visual Minteq and a simplified thermodynamic model) in order to determine the chemical compounds of interest. Experimental tests were then carried out with these compounds for assessing their flocculation ability. Both models revealed that precipitates of calcium phosphates and their substituted forms were the compounds involved in the auto-flocculation. Moreover, experimental tests showed that the stoichiometric neutralization of algal charges by calcium phosphates (i.e. hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$), octacalcium phosphate ($\text{Ca}_8\text{H}(\text{PO}_4)_6$) and amorphous calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$)), at a pH within the range 7–10 yields 70–82% recovered algal biomass. The optimum ratio required for algae auto-flocculation was 0.33 $\text{Ca}_5(\text{PO}_4)_3\text{OH}/\text{g DM}_{\text{algae}}$ at pH 10, 0.11 $\text{Ca}_8\text{H}(\text{PO}_4)_6/\text{g DM}_{\text{algae}}$ at pH 7 and 0.23 $\text{g Ca}_3(\text{PO}_4)_2/\text{g DM}_{\text{algae}}$ at pH 9. Auto-flocculation appears as a simple, sustainable and promising method for efficient harvesting of microalgae in a HRAP.

Keywords: high-rate algal pond (HRAP); microalgae; saturation index; auto-flocculation; calcium phosphates

1. Introduction

The high-rate algal pond (HRAP) is a biological treatment wastewater system based on the symbiosis between microalgae and heterotrophic photoautotrophic bacteria.[1, 2] Special conditions (i.e. low residence time, mechanical agitation and low depth) prevailing in the system, combined with the presence of light and nutrients, are the main elements that favour production of microalgae, which are useful in the field of bio-energy, the pharmaceutical industry, etc.[3–10] Their recovery in a HRAP is, however, considered to be a major technological and economic concern [3,11] due to their small size (3–30 μm diameter), their low density and their negative surface charge.[3,12–14] Although several methods for their harvesting have been successfully tested,[15–27] they were prohibitively expensive compared with the recovered products.[28]

In the context of algae recovery combined with wastewater treatment, it is desirable to recover these algae at low cost. Auto-flocculation could be attractive for achieving economic and environmental benefits because no addition of flocculants is required.[29] Auto-flocculation

presents the advantages of being simple and as effective as chemical flocculation, and could be an alternative to the problems encountered by conventional techniques.[29] Auto-flocculation is based on the reciprocal coagulation of microalgae by phosphate compounds such as apatites or substituted apatites.[30] Indeed, the photosynthetic activity of microalgae increases the pH of the medium and favours the precipitation of ions (e.g. phosphate, Ca^{2+}). These precipitated compounds coagulate microalgal cells, therefore leading to charge neutralization and the agglomeration of microalgae.[13]

Precipitation of calcium phosphates or magnesium hydroxide with algal cells was regularly quoted in the literature to be a mechanism inducing the auto-flocculation of microalgae.[8,30–33] However, very few experimental studies have been conducted for a more systematic verification of these mechanisms. This study aims to (1) identify the compounds involved in auto-flocculation through thermodynamic simulations and (2) assess using jar tests the flocculation potential of those compounds likely to precipitate with algal cells.

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2. Materials and methods

2.1. Thermodynamic simulation

Two models, using Visual Minteq 3.0 [34] and a simplified thermodynamic model,[30] were used to identify the compounds inducing auto-flocculation. The auto-flocculation potential was characterized through the saturation index (SI) of chemical compounds involved in algal suspension.

Visual Minteq 3.0 is a freeware chemical equilibrium model used mainly for the calculation of mineral speciation, solubility equilibrium and sorption of natural waters.[35] The aim of the programme is to simulate equilibrium and speciation of inorganic solutes in natural waters.

The simplified thermodynamic model has been developed in a previous study.[30]

2.1.1. Activity coefficient

The activity coefficient (γ_i) reflecting the interaction between species i and the other components of the system is calculated from Davies' equation [34]

$$\text{Log}(\gamma_i) = -A * Z_i^2 * \left(\frac{\sqrt{I_i}}{1 + \sqrt{I_i}} - 0.3 * I_i \right), \quad (1)$$

where $A \approx 0.509$ for water at 25°C,[36] Z_i is the valence of the ionic species and I_i corresponds to the ionic strength.

2.1.2. Ionic strength

The ionic strength (I_i) is shown in Equation (2).

$$I_i = 0.5 * \sum Z_i^2 * C_i, \quad (2)$$

where C_i refers to the molar concentration of ionic species during a phase of auto-flocculation.

2.1.3. Activity of species

The activities of ions in solutions were calculated from the concentrations of ions during auto-flocculation phases using simplified thermodynamic software [30] with Equation (3)

$$a_i = \gamma_i * C_i, \quad (3)$$

where a_i is the activity of ion i in the solution.

2.1.4. Saturation index

The SI (Equation (4)) of a compound $X_n Y_m$ was calculated as follows:

$$\text{SI} = \frac{\text{Log}(Q)}{\text{Log}(K_{\text{ps}})}, \quad (4)$$

where the product of ionic activities (Q) is calculated based on the measured activities of the various species under

HRAP conditions using the simplified thermodynamic software [30]

$$\text{Log}(Q) = a_{nX}^{m+} * a_{mY}^{n-}, \quad (5)$$

where K_{ps} is the solubility product for compounds at 25°C.

The saturation of the solution is determined according to the value of the SI: for $\text{SI} < 0$, the solution is under-saturated, for $\text{SI} = 0$, the solution is in equilibrium and for $\text{SI} > 0$, the solution is supersaturated; the more precipitation kinetics would be high.

2.2. Experimental investigation

2.2.1. Algal suspension

Dozens of samples (*Chlorella* (99.2%), *Chlamydomonas* (0.3%) and *Scenedesmus* (0.5%)) were collected in the HRAP at the end of exponential growth in order to obtain high algal concentrations and higher pH. The characteristics of the effluent correspond to the algal suspension arrived during this growth phase. This suspension contains: MES (380 mg L⁻¹), N-NH₄⁺ (48.6 mg L⁻¹), Ca²⁺ (90 mg L⁻¹), Mg²⁺ (25 mg L⁻¹), K⁺ (20 mg L⁻¹), Li⁺ (0.1 mg L⁻¹), Na⁺ (100 mg L⁻¹), Cl⁻ (100 mg L⁻¹), F⁻ (1 mg L⁻¹), N-NO₃⁻ (6,2 mg L⁻¹), S-SO₄²⁻ (50 mg L⁻¹), P-PO₄³⁻ (8.55 mg L⁻¹) and C-CO₃²⁻ (10 mg L⁻¹).

2.2.2. Measuring the optical density

The turbidity of the algal suspension was measured as the optical density (OD) at 695 nm using a spectrophotometer (UV/VIS Lambda 14, Perkin Elmer). Demineralized water was used as reference. After a settling period (30 min), the supernatant turbidity of samples was collected from beakers containing algal suspensions to measure the OD and to determine the recovery percentage.

2.2.3. Flocculation tests

The flocculation tests were conducted using a jar test [37] on the algal suspension and three flocculants (i.e. hydroxyapatite (Ca₅(PO₄)₃OH), octacalcium phosphate (Ca₈H(PO₄)₃) and calcium phosphate (Ca₃(PO₄)₂)). The methodology as described by Vandamme et al. [37] was used in our study.

The algal suspensions were divided into six replicate beakers of 600 mL. The initial algal biomass concentration in a beaker was estimated from the OD at 695 nm. The algal suspension pH is adjusted with sodium hydroxide (NaOH) (2 M) or hydrochloric acid (HCl) (3 M). Flocculants were added at a specific dose under intensive stirring (130 rpm) using a magnetic stirrer. After 5 min, the stirring speed was reduced to 40 rpm. Stirring was stopped at 30 min after addition of the reagents. After a further 30 min the OD of the supernatant was measured at half the height of the clarified layer.

The effect of the flocculants was assessed through the value of the flocculation efficiency (FE), calculated as follows:

$$FE = \left(\frac{OD_{t_0} - OD_t}{OD_{t_0}} \right) * 100, \quad (6)$$

where OD_{t_0} is the turbidity of sample at time t_0 and OD_t is the turbidity of the sample at time t .

2.2.4. Charge characterization

Two techniques (zeta potential [38–40] and colloidal titration [41]) were implemented experimentally to correlate particle charge to FE. However, due to the nature of the algal suspensions (the presence of different ions and

precipitated particles) and the presence of impurities, the zeta potential measurement led to less reproducible and unreliable results. Colloidal titration gave closer results. This is why colloidal titration was selected rather than zeta potential measurement.

The technique of colloid titration could be carried out in two ways: (1) a direct titration (e.g. calcium phosphates) with an oppositely charged polymer and (2) a back titration (e.g. microalgae) where a defined amount of an oppositely charged polymer is added to the solution to be investigated and the excess is then titrated.

Colloidal titration is based on the reaction between a cationic indicator, as *o*-toluidine blue (*o*-Tb) and an anionic polyelectrolyte as potassium vinyl sulphate (PPVS), which leads to a change in the absorption spectrum of

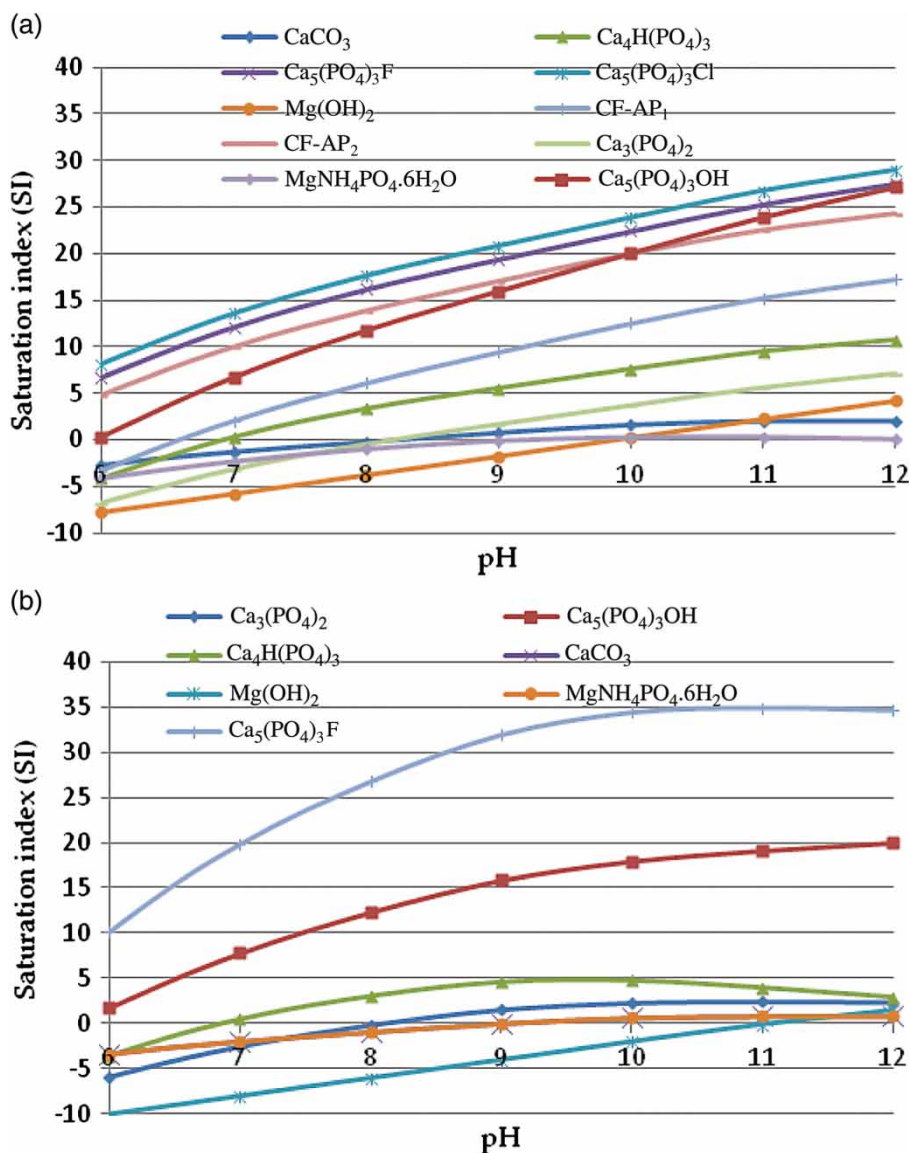


Figure 1. Evolution of SI of different chemical compounds using: (a) the simplified thermodynamic model and (b) Visual Minteq 3.0.

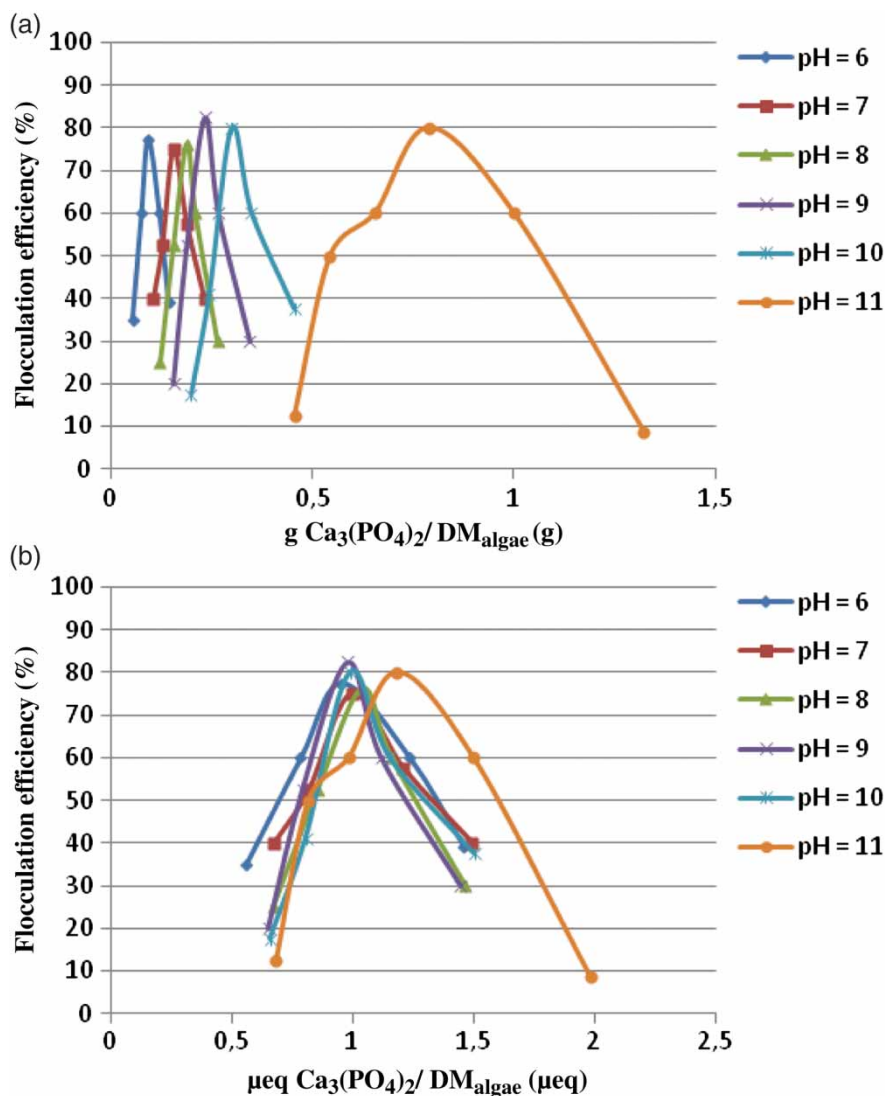


Figure 2. FE of $\text{Ca}_3(\text{PO}_4)_2$ at different pH (a) as a function of the mass ratio and (b) as a function of the charge ratio.

o-Tb and thus a visible colour change (blue to red-violet).[41]

2.2.5. Calcium phosphates

A defined amount of calcium phosphate was dissolved in 20 mL of pH buffered samples. The pH is adjusted with NaOH (2 M) or HCl (3 M) before being titrated with PPVS until the colour of the solution changed from blue to red-violet. The titration was repeated with a blank sample containing the same amount of indicator. Charge density (CD) of complexes was calculated as follows:

$$\text{CD} = \frac{\text{Charge}_{\text{titrant}} * \text{Volume}_{\text{titrant}} * C_{\text{titrant}} - \text{Charge}_{\text{blank}}}{m}, \quad (7)$$

where CD refers to charge density (meq g^{-1}), $\text{Charge}_{\text{titrant}}$ is the titrant solution charge (meq g^{-1}), $\text{Volume}_{\text{titrant}}$ the equivalent titrant volume (L), C_{titrant} is the titrant concentration

(g L^{-1}), $\text{Charge}_{\text{blank}}$ is the blank sample charge (meq) and m is the mass of the titrated solution (g).

2.2.6. Microalgae

Solutions for colloid titration were prepared by the addition of 25 mL of pH buffer to 100 mL of microalgae. The pH was adjusted with NaOH (2 M) or HCl (3 M). Poly diallyl dimethylammonium chloride polymer was added and the excess was titrated with PPVS. The microalgae CD could, therefore, be calculated (Equation (8))

$$\text{CD} = \frac{\text{Charge}_{\text{polycationic}} - (\text{Charge}_{\text{titrant}} + \text{Charge}_{\text{blank}})}{m}, \quad (8)$$

where $\text{Charge}_{\text{polycationic}}$ is the cationic polymer solution charge (meq), $\text{Charge}_{\text{titrant}}$ (meq), $\text{Charge}_{\text{blank}}$ (meq) and m (g).

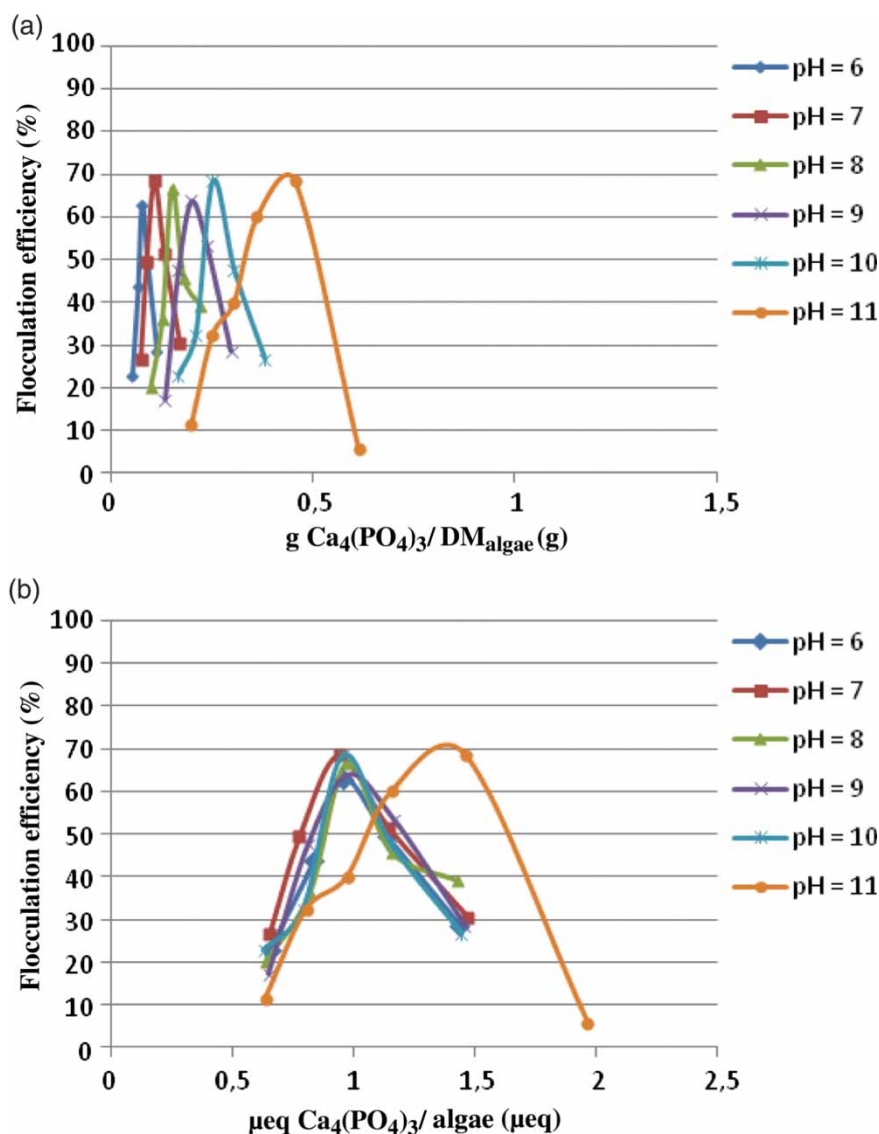


Figure 3. FE of $\text{Ca}_4\text{H}(\text{PO}_4)_3$ at different pH (a) as a function of the mass ratio and (b) as a function of the charge ratio.

3. Results

3.1. Calculation of SI

Figure 1 shows the evolution of SI of different chemical compounds in the algal suspension. The simulations carried out by the simplified thermodynamic model (Figure 1(a)) and Visual Minteq (Figure 1(b)) showed that calcium phosphates such as $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_4\text{H}(\text{PO}_4)_3$, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ and its substituted forms containing chlorine or fluorine compounds induced precipitation. These substituted forms were mainly fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$), chlorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{Cl}$), chlorofluoroapatite 1 ($\text{Ca}_5(\text{PO}_4)_2.5(\text{CO}_3)_{0.5}\text{F}_{1.36}(\text{OH})_{0.14}$) and chlorofluoroapatite 2 ($\text{Ca}_{4.658}\text{Mg}_{0.072}\text{Na}_{0.18}(\text{PO}_4)_{2.4}(\text{CO}_3)_{0.6}\text{F}_{1.24}$).

The evolution of the SI of the precipitated compounds, using the two models, was almost similar for a pH ranging

between 6 and 8. Differences were recorded for pH values greater than 8 (Figure 1).

Calcium phosphates and its substituted forms had the greatest SI (between -10 and 35) relating to the pH. Furthermore, compounds such as hydroxides $\text{Mg}(\text{OH})_2$ and struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) showed a negative or very low SI (Figure 1). Based on the thermodynamic characteristics of our experimental environment, these compounds were very unlikely to precipitate.

3.2. Effect of calcium phosphates on microalgae auto-floculation

Figures 2–4 show the effects of the three main calcium phosphates ($\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_4\text{H}(\text{PO}_4)_3$ and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$) on microalgae auto-floculation. The FE at different pHs (e.g. from 6 to 11) significantly increased in a straight

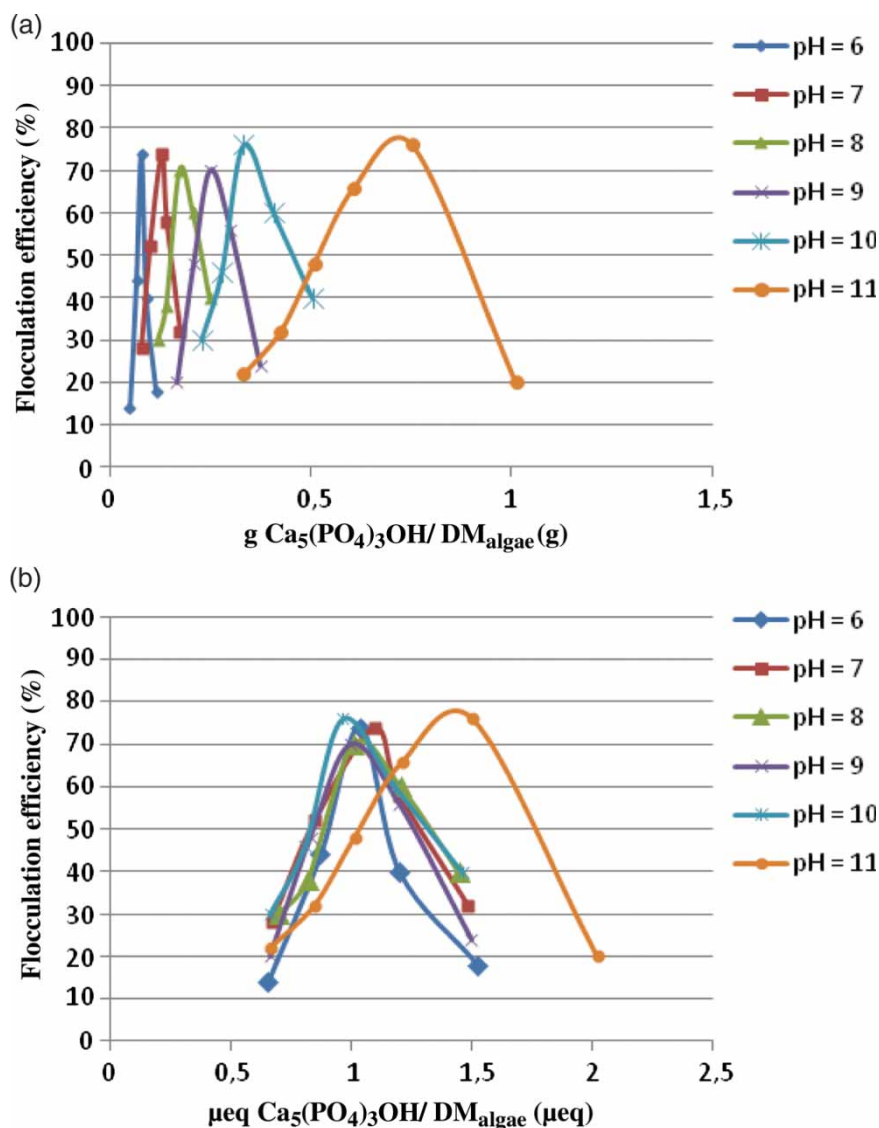


Figure 4. FE of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ for different pH (a) as a function of the mass ratio and (b) as a function of the charge ratio.

range of mass ratio between calcium phosphates and algae (approximately 0.05–0.5 g calcium phosphates by gram of algae dry matter (DM_{algae})). In addition, the charge ratio required to achieve this FE was 1:1 at pH 6–10 and was higher than 1 at pH 11 (e.g. $\text{Ca}_4\text{H}(\text{PO}_4)_3$ and $\text{Ca}_5(\text{PO}_4)_3\text{OH}$).

3.2.1. Effect of $\text{Ca}_3(\text{PO}_4)_2$

About 82% of the microalgae was harvested at pH 9 and a mass ratio of 0.23 g $\text{Ca}_3(\text{PO}_4)_2/\text{g DM}_{\text{algae}}$ (Figure 2(a)). The floculation reaction was stoichiometric (1:1) in terms of charge compensation at a pH ranging between 6 and 11 (Figure 2(b)).

3.2.2. Effect of $\text{Ca}_4\text{H}(\text{PO}_4)_3$

At pH 7, 70% of microalgae was harvested for a mass ratio of 0.11 g $\text{Ca}_4\text{H}(\text{PO}_4)_3/\text{g DM}_{\text{algae}}$ (Figure 3(a)). The charge

ratio required to achieve the most effective floculation of microalgae was approximately 1 $\mu\text{eq Ca}_4\text{H}(\text{PO}_4)_3/\mu\text{eq}$ algae at pH 6 to 10 (Figure 3(b)), whilst at pH 11 this ratio was 1.46 $\mu\text{eq Ca}_4\text{H}(\text{PO}_4)_3/\mu\text{eq}$ algae.

3.2.3. Effect of $\text{Ca}_5(\text{PO}_4)_3\text{OH}$

A ratio of 0.33 g $\text{Ca}_5(\text{PO}_4)_3\text{OH}/\text{g DM}_{\text{algae}}$ induced 76% of microalgae floculation at pH 10 (Figure 4(a)). The most effective microalgae harvesting was obtained for a charge ratio of 1 $\mu\text{eq Ca}_5(\text{PO}_4)_3\text{OH}/\mu\text{eq}$ algae for pH 6–10, and 1.51 $\mu\text{eq Ca}_5(\text{PO}_4)_3\text{OH}/\mu\text{eq}$ algae at pH 11 (Figure 4(b)).

4. Discussion

Our study was aimed at identifying the compounds involved in auto-floculation by thermodynamic simulations. The

two models used for the simulations have shown that the microalgal solution was initially saturated in calcium phosphates and its substituted forms, according to their positive SI. These compounds are consequently most likely to be involved in the auto-flocculation of microalgae. These results are in accordance with the studies by Zawacki et al., [42] Heughebaert et al. [43] and Heubeck et al., [44] who showed that, in complex environments, both pure apatites and substituted forms precipitated. Furthermore, these compounds, positively charged, induce the flocculation of microalgae (which are negatively charged). [16, 19, 21, 30, 31, 41, 42, 45] The positive charge of the precipitate attracts the negatively charged microalgae cells, resulting in the compression of the potential to the limit of the stern layer and causing them to become destabilized and hence to flocculate.

The study also emphasized the flocculation ability of the chemical compounds likely to precipitate with microalgae. The optimum mass ratio to reach efficient recovery of microalgae depends on pH (0.33 $\text{Ca}_5(\text{PO}_4)_3\text{OH}/\text{g DM}_{\text{algae}}$ at pH 10, 0.11 $\text{Ca}_4\text{H}(\text{PO}_4)_3/\text{g DM}_{\text{algae}}$ at pH 7 and 0.23 $\text{g Ca}_3(\text{PO}_4)_2/\text{g DM}_{\text{algae}}$ at pH 9). Our result indicates that the three calcium phosphates tested are efficient flocculants for microalgae harvesting. In addition, the reaction is stoichiometric in the sense of 1:1 charge compensation. Thus, the concentration of flocculants needed to reach microalgae flocculation is very narrow and follows a stoichiometric relationship. Therefore, this is the surface developed by the particle counts. The flocculant dose required for optimal flocculation could be related to the surface charge of algal cells and can be determined on this basis.

5. Conclusion

This study highlights the natural mechanisms involved in microalgae auto-flocculation. Calcium phosphates and its substituted forms were identified as compounds inducing the auto-flocculation in a HRAP. The precipitation of calcium phosphates and its substituted forms coagulate with microalgae and lead to algal stoichiometric charge neutralization, thereby inducing auto-flocculation. Compared with chemical flocculation, no addition of flocculants was necessary to reach auto-flocculation in a HRAP. In fact, as Ca^{2+} and PO_4^{3-} are naturally present in wastewater, the HRAP can be operated to reach the optimum pH at these concentrations to engage auto-flocculation. It is, therefore, interesting to consider auto-flocculation for reducing harvesting costs. Auto-flocculation appears to be a simple, sustainable and promising method for an efficient harvesting of microalgae in a HRAP. This study could serve as a basis for an optimal management of reactors. Biological reactor modelling is certainly a useful tool for this purpose. Further research could also be undertaken for a better understanding of the kinetics involved in this process.

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