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# Physicochemical approach to alkaline flocculation of *Chlorella vulgaris* induced by calcium phosphate precipitates

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## ABSTRACT

Alkaline flocculation has been studied due to its potential as a low-cost harvesting method for microalgae. However, surface properties (zeta potential, contact angles) as inputs into physicochemical interaction models have not yet been applied systematically. In this work, forced alkaline flocculation of the freshwater microalgae *Chlorella vulgaris* induced by calcium phosphate precipitates was studied as a model system. Response surface methodology was used to quantify the effect of independent variables (concentration of $Ca^{2+}$ (0.5–0.5 mM) and $PO_4{}^{3-}$ (0.05–0.35 mM), pH (8–12) and ionic strength (1–19 mM)) on the zeta potential (ZP) of microalgae, and the turbidity (T) of inorganic precipitates. Flocculation tests and their modified versions were carried out. The flocculation efficiencies obtained were interpreted with respect to predictions of physicochemical interaction models. It was found that flocculation was possible under conditions where appropriate precipitates were formed in the presence of cells. Under these conditions, flocculation of negatively charged *Chlorella vulgaris* was induced not only by positively charged, but also by negatively charged calcium phosphate precipitates at an early phase of nucleation. The driving force for interactions between oppositely charged cells and precipitate particles was electrostatic attraction, while the attraction between equally charged entities may have resulted from a negative total balance of apolar (Lifsitz-van der Waals) and polar (acid-base) interactions. Medium components did not interfere with flocculation, while cellular organic matter decreased flocculation efficiency only to a very limited extent.

## 1. Introduction

Microalgae have high areal productivity [1] and contain valuable compounds [2]. However, the production costs of microalgae restrict their application to only high-value products. To improve the economic feasibility of microalgal biotechnologies, production costs must be reduced significantly. Major savings can be achieved by cost-efficient methods of harvesting, since separation of microalgae can represent 20–30% of the overall production costs [3]. Centrifugation, as used for high-value products, is questionable for low value-added products such as biofuels. The reason lies in small cell size and low biomass concentration of microalgal cultures (0.5–5 g $L^{-1}$). Under these conditions, centrifugation is expensive because of the high investment and operational costs [3]. Economics of microalgal harvesting can be improved by flocculation methods, which are usually used for pre-concentrating microalgal suspensions [4]. In addition, flocculation seems to lower the operating costs of centrifugation as well as facilitating easy filtration [5].

Since the terminology concerning microalgal flocculation is somewhat misleading, this article will use the following classification of different flocculation methods: i) spontaneous and forced alkaline flocculation [6], ii) chemical flocculation with addition of flocculants [7], iii) autoflocculation provoked by extracellular polymeric substances (EPS) [8], and iv) bioflocculation, which involves other microorganisms [9].

Among flocculation methods, high pH induced (alkaline) flocculation of algae, mediated by inorganic salt precipitates has the advantage of using cheap hydroxides (e.g. slaked lime) instead of chemical flocculants [10]. Flocculation induced by both a natural

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**Table 1**
Independent variables, their coded levels and actual values.

| Symbol | Independent variables (units) | Coded level | | |
|---|---|---|---|---|
| | | −1 | 0 | +1 |
| Ca | $Ca^{2+}$ (mM)[a] | 0.5 | 3.0 | 5.5 |
| P | $PO_4^{3-}$ (mM)[a] | 0.05 | 0.20 | 0.35 |
| pH | pH[a,b] | 8 | 10 | 12 |
| IS | Ionic strength (mM)[b] | 1 | 10 | 19 |

[a] Variables for characterization of CaP precipitates.
[b] Variables for characterization of *C. vulgaris* cells.

**Table 2**
Central composite design matrix and response values of average zeta potentials (ZP) of *C. vulgaris* cells and precipitate particles and turbidities (T) of CaP precipitate particles as a result of variations in $Ca^{2+}$ (Ca) and $PO_4^{3-}$ (P) concentrations, ionic strength (IS) and pH.

| Runs | Variables | | | | Responses | | |
|---|---|---|---|---|---|---|---|
| | Ca[a] | P[a] | IS[b] | pH[a,b] | ZP[a] [mV] | T[a] [FTU] | ZP[b] [mV] |
| 1 | −1 | −1 | −1 | −1 | −4.4 | 0.12 | −34.4 |
| 2 | +1 | −1 | +1 | −1 | 10.3 | 0.48 | −19.2 |
| 3 | 0 | 0 | 0 | −1 | 5.3 | 0.60 | −22.0 |
| 4 | −1 | +1 | – | −1 | −1.5 | 0.72 | – |
| 5 | +1 | +1 | – | −1 | 9.7 | 1.68 | – |
| 6 | 0 | −1 | +1 | 0 | 10.1 | 1.32 | −20.7 |
| 7 | −1 | 0 | −1 | 0 | −6.5 | 3.88 | −38.2 |
| 8 | 0 | 0 | 0 | 0 | 10.4 | 2.64 | −24.3 |
| 9 | +1 | 0 | 0 | 0 | 11.1 | 3.00 | −23.8 |
| 10 | 0 | +1 | – | 0 | 9.2 | 5.80 | – |
| 11 | −1 | −1 | +1 | +1 | −9.1 | 1.56 | −21.8 |
| 12 | +1 | −1 | −1 | +1 | 9.3 | 1.12 | −36.9 |
| 13 | 0 | 0 | 0 | +1 | 4.3 | 1.76 | −23.5 |
| 14 | −1 | +1 | – | +1 | −26.8 | 7.76 | – |
| 15 | +1 | +1 | – | +1 | 6.5 | 4.08 | – |

[a] Variables and responses for precipitates.
[b] Variables and response for *C. vulgaris*.

increase in pH due to $CO_2$ depletion [11,12] and addition of magnesium/calcium hydroxide [13,14] has long been reported and various mechanisms have been suggested. Neutralization of the surface charge of microalgae by oppositely charged colloidal precipitates is the most common mechanism [11]. Meantime, another mechanism called entrapment of cells by precipitates (sweeping flocculation) was hypothesized [15,16].

Due to the small size of interacting entities, alkaline flocculation of algal cells and inorganic salt precipitates can be examined using thermodynamic balance of free energies [17] and colloidal Derjaguin-Landau-Verwey-Overbeek (DLVO) interaction theory [18]. The thermodynamic approach considers the stability of interactions but omits the role of long range electrostatic interactions [19]. The DLVO approach describes the interaction between surfaces on the basis of Lifshitz-van der Waals (LW) and electrostatic (EL) [20]. Agreement between models and experimental data can aid in understanding the flocculation mechanism and, subsequently, in optimizing process steps leading to reliable alkaline flocculation.

So far there has been no colloidal interaction model applied to demonstrate the role of physicochemical interactions in forced alkaline flocculation of the biotechnologically attractive freshwater microalgae *Chlorella vulgaris*. Therefore, this paper presents experimental data on the importance of surface charge, surface tension and the size of interacting particles, environmental conditions (pH, ionic strength), medium components and cellular organic matter on cell-precipitate interactions.

## 2. Material and methods

### 2.1. Microorganism, cultivation and preparation of algal suspension

*Chlorella vulgaris* Beijerinck, strain CCALA 256 was isolated in New Zealand (1982) and deposited in the Culture Collection of Autotrophic Organisms (CCALA) in Trebon, Czech Republic (http://ccala.butbn.cas.cz/en/). Batch cultivation was carried out according to Prochazkova et al. [18]. After 168 h of cultivation a biomass concentration of $6\,g\,L^{-1}$ was obtained, then the microalgal cells were centrifuged (2865 × g, 5 min, 22 °C) and washed twice with distilled water. Subsequently, they were used for image analysis, zeta potential measurements and flocculation studies.

### 2.2. Experimental design and response surface methodology

Response surface methodology (RSM) was used to quantify the effect of three independent variables ($Ca^{2+}$ (Ca), $PO_4^{3-}$ (P) and pH) on zeta potential (ZP) and turbidity (T) of inorganic precipitates. Subsequently, the effect of ionic strength (IS) and pH on ZP of *C. vulgaris* cells was determined. The variables were changed over three levels (Table 1) and optimized using a small central composite design, based on a two-level factorial design (alpha face centred). A total of 15 and 10 experiments were used to investigate the ZP of precipitates and cells, respectively. Additionally, 15 experiments were carried out to obtain the effects on turbidity of precipitates (Table 2). Experimental design and multiple regression analyses of the experimental data were performed using Design Expert software (version 9.0.4.1, Stat-Ease Inc., MN, USA). The experimental data were statistically evaluated using ANOVA. The fitted polynomial equation was visualized in the form of 3D surface plots to help navigate in the design space.

### 2.3. Zeta potential measurement

Samples of precipitates were prepared by dissolving the appropriate amounts of $CaCl_2$ and $KH_2PO_4$ in deionized water (250 mL) and the pH was adjusted to 8–12 by KOH (1 M). The ZP of the solutions was measured after agitation with a magnetic stirrer (1.5 cm in length, 200 rpm, 25 °C). ZP values obtained after 5 min were within ±5% of the final value and thus were used for evaluation by RSM.

In order to avoid the flocculation of microalgae, which would make ZP measurements impossible, the suspension of cells was prepared in a symmetrical electrolyte (KCl) with ionic strengths (1–19 mM) and pH corresponding to solutions used for the ZP measurement of precipitates.

Zeta potentials of *C. vulgaris* cells and calcium phosphate (CaP) precipitates were measured at 25 °C using the Zetasizer Nano-ZS (Malvern, United Kingdom) and calculated according to the Helmholtz-Smoluchowski equation [21]. Each sample was measured at least three times. Results are presented as mean values with an experimental error of ±5%.

### 2.4. Turbidity and size measurement

Samples of precipitates were prepared by dissolving the appropriate amounts of $CaCl_2$ and $KH_2PO_4$ in deionized water (250 mL) and adjusting the pH with KOH (1 M). The turbidity (T) of the solutions was measured after agitation with a magnetic stirrer (1.5 cm in length, 200 rpm) for 5 min (25 °C) with a laboratory hazemeter MZN-93-MC2 (Charles University, Czech Republic). The measurements were done at an angle of 15°. The same samples were used to measure precipitate particle size at 25 °C using the Zetasizer Nano-ZS (Malvern, United Kingdom). All experiments were performed in triplicate and results are presented as mean values with an exper-