Synergies of pH-induced calcium phosphate precipitation and magnetic separation for energy-efficient harvesting of freshwater microalgae

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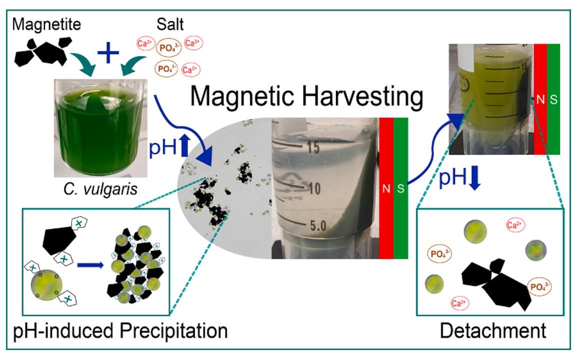
GRAPHICAL ABSTRACT



ABSTRACT

Keywords:
Magnetic separation
Microalgae harvesting
Magnetite microparticles
Flocculation
pH-induced precipitation

Energy- and time-consuming concentration steps currently limit the industrial application of microalgae. Compared to state-of-the-art technologies, magnetic separation shows a high potential for efficient harvesting of microalgae. This study presents a novel approach to combine pH-induced calcium phosphate precipitation with cheap natural magnetite microparticles for magnetic separation of the freshwater microalgae *Chlorella vulgaris*. Harvesting efficiencies up to 98% were achieved at moderate pH and low particle and calcium phosphate concentrations in a model medium. However, cultivation-dependent high loads of algogenic organic matter can severely inhibit flocculation and particle/algae interactions, requiring higher salt concentrations or pH. Harvesting efficiencies above 90% were still attainable at moderate pH with increased calcium phosphate concentrations of 10 mM. Acidification of the suspension to pH 5 allows for simple and reversible particle recycling. The presented process provides a promising path to universal and cost-effective harvesting, advancing the utilization of microalgae as a sustainable bioresource.

1. Introduction

Growing emphasis on environmental footprint and sustainable production processes has increased interest in renewable bioresources. Unlike conventional bioresources, agricultural soils are not required in case of microalgae cultivation. In addition, microalgae provide higher growth rates and photosynthetic activity with lower water demand (Alam et al., 2020). Their high CO_2 fixation capacity of about 1.83 kg per

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kg biomass also provides an opportunity for decarbonization. The potential of microalgae for the cost-effective production of biofuels, food/feed, bioplastics, and pharmaceuticals presents a promising path toward reducing reliance on fossil raw materials (Fresewinkel et al., 2014). However, due to the small cell sizes and the dilute nature of microalgae cultures ($< 2\,g\,L^{-1}$), separation and dewatering steps are major challenges that limit their industrial application (Vanthoor-Koopmans et al., 2013). Harvesting is very time and energy-intensive and accounts for 20 - 30% of the total production costs, often resulting in negative cost balances for many applications (Uduman et al., 2010). Currently, there is no universal cost-effective harvesting method suitable for all algae types and applications. The current state-of-the-art method for microalgae harvesting is centrifugation. In more advanced processes, a preliminary thickening step using sedimentation or flocculation is employed to improve economic viability. Nonetheless, even these procedures entail significant energy consumption, with specific energy utilization averaging around $1\,kWh/m^3$ in optimized systems (Schlagermann et al., 2012).

Magnetic separation systems offer high efficiency at lower operating costs (Franzreb et al., 2006). When applying magnetic separation for algae harvesting, biocompatible magnetic particles bind to the microalgae and are separated or retained by magnetic forces. The particle/microalgae interaction is primarily influenced by cell surface components, such as polysaccharides and adhesion proteins, as well as electrostatic interactions and charge neutralization phenomena (Wang et al., 2015; Procházková et al., 2012). A potential model for a first estimation of the binding energy of synthesized particles on microalgae in a model medium is the XDLVO model (Prochazkova et al., 2013). The use of low-cost magnetic microparticles (MMPs) larger than 1 µm, instead of the commonly studied magnetic nanoparticles between 10 and 100 nm in size (Kim et al., 2013; Lee et al., 2014; Safarik et al., 2016; Berensmeier, 2006; Laurent et al., 2010), facilitates efficient high-throughput separation using permanent magnet-driven separators instead of electromagnetically operated high-gradient magnetic separators. This approach enhances energy efficiency and guarantees a process with a more favorable carbon footprint. In particular, using naked iron oxide (magnetite) particles can increase the profitability of magnetic microalgae harvesting (Xu et al., 2011). Prochazkova et al. (2013) reported separation efficiencies over 95% for harvesting *Chlorella vulgaris* from highly diluted suspensions utilizing microwave-treated inexpensive iron oxide MMPs. However, due to lower specific surface areas, higher particle/algae ratios are often required for comparable harvest efficiencies with MMPs.

Combining magnetic separation with flocculation-based processes can address this disadvantage. In conjunction with other methods, flocculation processes gained increasing interest due to their high efficiency and scalability (Enamala et al., 2018; Milledge et al., 2013; Lama et al., 2016). However, high toxicity and other undesirable effects prevent the usage of conventional chemical flocculants, such as aluminum sulfates or ferric chloride, in food, feed, or pharmaceutical applications (Sharma et al., 2013; Ummalyma et al., 2016; Matter et al., 2019). pH-induced flocculation represents an alternative low-energy approach that utilizes decreasing solubilities of added calcium and magnesium salts (Vandamme et al., 2015). The resulting precipitates form preferentially on the particles and algae (heterogeneous nucleation) and lead to the formation of flocs/agglomerates, which are easily magnetically separable. For efficient harvesting, Cerff et al. (2012) suggested separation at high pH and in the presence of divalent and trivalent ions, which results in the flocculation of algae around the entrapped magnetic particles. Highly alkaline conditions are commonly required for sufficient harvesting efficiency and reproducibility. For saline microalgae, efficient separation is possible without adding salts due to the high amounts of magnesium in the medium (Schobesberger et al., 2021). However, harvesting freshwater algae usually requires the addition of salts (Beuckels et al., 2013; García-Pérez et al., 2014). Compared to magnesium hydroxide precipitation occurring at a pH above 10.5, the addition of calcium phosphate salts to the suspension enables fast and efficient separation at moderate pH (pH 7 - 10), which can be achieved by simple photosynthetic CO_2 depletion (Sukenik et al., 1984).

Even small amounts of cultivation-dependent algogenic organic matter (AOM) strongly inhibit flocculation and require increased flocculant dosages or pH (Roselet et al., 2017; Beuckels et al., 2013; Vandamme et al., 2016), with pH-induced flocculation being the least affected (Vandamme et al., 2012). Thus, to prevent stress-induced secretion of AOM, the cultivation conditions of microalgae have to be highly regulated. In addition, cultivation time can also have a significant influence, as AOM is mainly formed during the stationary growth phase of microalgae (Rashid et al., 2019).

To date, there is a lack of studies on the magnetic separation of freshwater microalgae at moderate pH. Furthermore, despite the growing interest in algal harvesting by methods such as flocculation and magnetic separation, there are few studies examining the potential synergistic benefits of combining these techniques even though this has the potential to become a universal and cost-effective harvesting method. Moreover, the influence of AOM on MMP-mediated harvesting has received limited attention in the existing literature. In addition, while some publications (Cerff et al., 2012; Xu et al., 2011; Prochazkova et al., 2013) have indicated the need to address particle detachment and recovery, comprehensive investigations in this regard have been limited (Fraga-García et al., 2018; Prochazkova et al., 2013).

This study presents a novel approach to utilize the synergies of pH-induced calcium phosphate precipitation with natural MMPs for fast and efficient magnetic separation of the freshwater microalgae *C. vulgaris*. Small-scale screening experiments were performed in a model medium containing 10 mM NaCl to investigate the influence of individual ions, such as calcium and phosphate, as well as their combination on magnetic microalgal harvesting at moderate pH. Furthermore, the impact of high loads of cultivation-dependent AOM on flocculation and the particle/algae interaction was investigated to provide a more holistic understanding of the complex factors that affect magnetic separation. In addition, this study demonstrates how pH-induced salt precipitation facilitates efficient particle recycling.

2. Material and methods

2.1. Magnetite microparticles

The MMPs (EX009) were kindly provided by LKAB Minerals GmbH (LKAB: Luossavaara-Kiirunavaara Aktiebolag), Germany. The natural iron ore particles were mined in Malmberget, Sweden. The MMPs were stored as a dry powder (as supplied) and suspended in culture just before algal separation.

The size of the MMPs was determined by static light scattering (SLS) (Partica LA-950, Horiba Europe GmbH, Germany). The specific surface area of the particles was calculated from BET isotherms of nitrogen adsorption (Gemini VII, Micrometrics, Germany). Scanning electron microscopy (SEM) images were obtained with a Philips XL30 ESEM (Philips, Netherlands) operating at 20 kV acceleration voltage. Prior to measuring, samples were sputtercoated with a 5 nm conductive platinum layer using a BAL-TEC MED 020 (BAL-TEC AG, Liechtenstein). The magnetization properties of the MMPs were measured using an alternating gradient magnetometer (MicroMag 2900, Lake Shore Cryotronics, USA). For the measurement, thin glass capillaries were filled with particle powder and sealed. The field increment was set to 3.18 kA/m while the measurement points were averaged every 300 ms. The measurement was normalized by sample mass.

2.2. Microalgae

C. vulgaris (strain 211-11b SAG, Germany) was used as model species for the investigation. The microalgae cells were cultivated in 1 L Erlenmeyer flasks with 400 mL BG11-medium ($1.5\,g\,L^{-1}$ $NaNO_3$, $0.39\,mg\,L^{-1}$

$Na_2MoO_4 \cdot 2H_2O$, 0.02 g L ^{1} Na_2CO_3, 0.075 g L ^{1} $MgSO_4 \cdot 7H_2O$, 0.036 g L ^{1} $CaCl_2 \cdot 2H_2O$, 0.04 g L ^{1} KH_2PO_4, 1 mg L ^{1} $Na_2EDTA \cdot 2H_2O$, 2.86 mg L ^{1} H_3BO_3, 0.22 mg L ^{1} $ZnSO_2 \cdot 7H_2O$, 1.81 mg L ^{1} $MnCl_2 \cdot 4H_2O$, 0.08 mg L ^{1} $CuSO_2 \cdot 5H_2O$, 6 mg L ^{1} ammonium iron(III) citrate, 6 mg L ^{1} citric acid, 0.05 mg L ^{1} $Co(NO_3)_2 \cdot 6H_2O$ and adjusted to pH 7.1) on a horizontal shaker at a speed of 130 rpm. The cultures were under continuous illumination at a light intensity of 100 µmol m ^{2}s ^{1} and aerated at 0.15 vvm with a mixture of air and 1% CO_2. The initial biomass concentration of the cultures was 0.1 g L ^{1}. Culture growth was monitored by measuring optical density at 750 nm in a Spark multimode microplate reader (Tecan, Switzerland) and correlated to gravimetrical measurements of biomass dry weight. The studies were performed on microalgal cells at the beginning of the stationary phase (9-11 days). The final microalgae concentration after cultivation was 2.1 g L ^{1}.

The zeta potential of the microalgae suspensions was measured at 25 °C using a Zetasizer (Malvern Instruments, UK). The amount of total organic carbon (TOC) after cultivation in the supernatant was measured with a TOC analyzer (Sievers 900 Laboratory TOC Analyzer, GE Healthcare). TOC was employed as a sum parameter for the initial assessment of the cultivation-dependent AOM load in the culture and the determination of appropriate particle and calcium phosphate concentrations for subsequent separation.

2.3. Separation experiments

Separation experiments were performed in 50 mL centrifuge tubes. Preliminary interaction experiments were conducted with 1 g L ^{1} *C. vulgaris* in BG11-medium with particle/algae ratios between 1-10 $g_{MMP}\, g_{algae}^{1}$ at acidic, moderate and basic conditions. Calibration curves to relate optical density of the microalgae culture and gravimetric measurements of biomass dry weight were employed to determine the precise particle dosage needed to achieve specific particle/algae ratios. Furthermore, the influence of high ionic strength on the interaction was assessed by adding 0.1 M or 0.5 M NaCl to the suspension. Following experiments utilized particle/algae ratios of 1 $g_{MMP}\, g_{algae}^{1}$ to further explore potential factors that could improve the interaction. To investigate the influence of calcium and phosphate on the interaction, *C. vulgaris* cultures were centrifuged, washed three times with deionized water, and resuspended to 1 g L ^{1} in a model medium containing 10 mM NaCl. The influence of the medium salts on the separation was investigated by adding 1.5 mM and 10 mM of the respective ions (phosphate and calcium) and compared with control solutions without additional ions. Above a critical pH, pH-induced flocculation may occur due to precipitation of calcium phosphate salts as a result of lower solubility. Thus, flocculation influence on magnetic algae separation was investigated by pH variation between pH 4 and 10.

The choice of calcium and phosphate concentrations for effective flocculation at moderate pH initially relied on existing literature (Beuckels et al., 2013). However, due to the pronounced inhibitory effects of high concentrations of AOM in the original culture medium, higher flocculant dosages were applied. Flocculation and separation inhibition by growth-dependent AOM was determined by separation experiments in the original culture solution. Several concentrations of phosphate and calcium salts were added, including stoichiometric amounts for hydroxyapatite. pH was varied between 7 and 10 since salt precipitation and flocculation in the model medium were only observed above pH 7.

For the experiments, 15 mL particle/algae suspensions were intensively mixed during and immediately after pH adjustment by the addition of 1 M HCl or 1 M KOH. Each separation was performed with an MMP concentration of 1 $g_{MMP}\, g_{algae}^{1}$. The suspensions were then incubated with gentle shaking at 80 rpm on an orbital shaker for 10 min. Afterwards, the suspensions were magnetically separated for 5 min using a NdFeB permanent magnet (Webcraft, Germany). The supernatant was examined using UV–vis (Spark, Tecan, Switzerland) to assess the binding efficiency of the MMPs on microalgae. Sedimentation of the algae was neglected due to the short separation time. Microalgae harvesting efficiency was calculated by the following equation:

$$Harvesting\ efficiency\ = \frac{OD_{750nm,0} \quad OD_{750nm}}{OD_{750nm,0}} \cdot 100\,\% \tag{1}$$

where $OD_{750nm,0}$ corresponds to the optical density of the supernatant at 750 nm before the addition of particles and OD_{750nm} after magnetic separation. All experiments were performed as triplicates unless otherwise specified.

For further analysis of the salt concentrations before and after separation, inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements of the supernatant composition were performed using a Perkin Elmer Optima 8300 DV (Perkin Elmer, USA). Optical and fluorescence microscope images of the algae/MMP composites were obtained using an Axio Observer Z1 (Carl Zeiss AG, Germany).

2.4. Detachment experiments

For the detachment experiments, preceding separation experiments were performed with 30 mL particle/algae suspensions (1 $g_{MMP}\, g_{algae}^{1}$) at pH 8.5 (see Chapter 2.3). Medium (5 mM Ca and 3 mM PO_4) and high (10 mM Ca and 6 mM PO_4) dosages of flocculant were added to compensate for inhibition by the culture solution. In addition, 0.1 mM and 0.5 mM NaCl were added to the high-dosed suspensions to investigate the influence of ionic strength on separation, flocculation, and recycling. Prior to magnetic separation, particle/algae flocs were allowed to settle for 5 min to determine the sludge volume. The suspensions were then gently mixed to eliminate sedimentation influences on separation efficiency. Following magnetic separation, the solution was adjusted to pH 5 by adding 1 M HCl and the particle/algal sludge was resuspended. Subsequently, the sludge volumes and separation efficiencies were determined again after 5 min of sedimentation, gentle mixing, and magnetic separation, respectively. Particles were magnetically separated from the culture solution, washed three times with deionized water, and their dry weight was measured gravimetrically to evaluate particle recovery after each detachment experiment.

3. Results and discussion

Although the interaction of magnetic particles with microalgae, such as *C. vulgaris*, has been the subject of several studies, the exact phenomena are not fully understood, particularly in the case of MMPs (Prochazkova et al., 2013; Schobesberger et al., 2021). Besides the amounts of particles, cultivation media and conditions were reported as two major factors influencing the interaction, as they affect cell surface properties and the presence of promoting or inhibiting substances in the medium (Cerff et al., 2012; Branyikova et al., 2018). This section first provides data characterizing the used natural MMP and show the influence of medium-associated salt ions and high loads of stress-induced AOM on magnetic microalgae separation in model medium and culture solution, respectively. Furthermore, the study demonstrates how pH-induced salt precipitation simplifies efficient particle recycling.

3.1. Influence of the magnetic microparticles

The magnetite particles used in this study were milled by LKAB Minerals GmbH after mining and have a rather narrow particle size distribution ($d_{10} = 4.66$ µm, $d_{50} = 5.51$ µm, $d_{90} = 6.51$ µm). The shape of the particles is edged and appears cubic rather than spherical (see supplementary material). In addition, a small amount of fine fraction (< 1 µm) was observed, likely due to the grinding process. The specific surface area of the MMPs calculated from BET-isotherms was 2.39 $m^2 g^{1}$. The high saturation magnetization of 89.6 $Am^2 kg^{1}$ and remanence of 20.4 $Am^2 kg^{1}$ indicate a high purity of magnetite with low maghemite content and good magnetic behavior (Schwaminger et al., 2017).

The interaction between particles and microalgae is a complex phenomenon influenced by various factors. Preliminary separation experiments of $1\,g\,L^{-1}$ *C. vulgaris* in BG11-medium highlight the importance of a sufficient particle/algae ratio for achieving high separation performance (see Fig. 1 (a)). Applying ratios between $1 - 10\,g_{MMP}\,g_{algae}^{-1}$ at different pH values resulted in separation efficiencies ranging from 46% to 96%. These results indicate a good affinity of the selected particles for

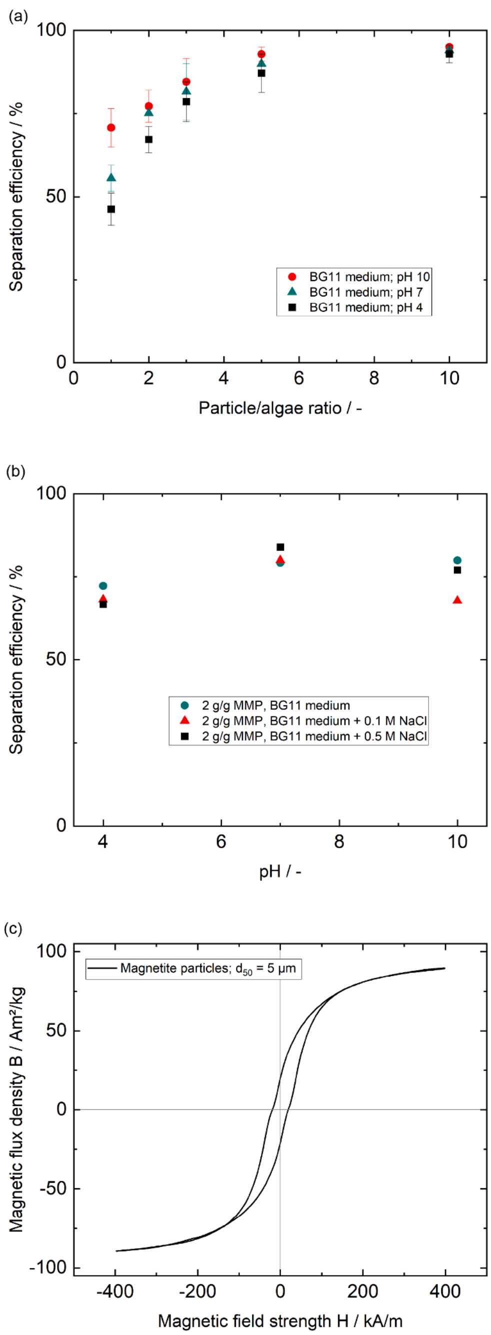

Fig. 1. Influence of the natural magnetite microparticles EX009 on the magnetic separation of $1\,g\,L^{-1}$ *C. vulgaris* in BG11-medium. Influence of particle/algae ratio (a) and ionic strength (b) on the harvesting efficiency at acidic, moderate, and basic pH conditions. Ionic strength experiments were performed in duplicate, and the mean values of the separation efficiency are visualized. (c) Hysteresis loop of EX009 showing a saturation magnetization of $89.6\,Am^2\,kg^{-1}$ and a remanence of $20.4\,Am^2\,kg^{-1}$.

C. vulgaris in BG11-medium, suggesting that the specific surface area may be a limiting factor in the separation process. Fraga-García et al. (2018) reported similar mass ratios and efficiencies in the separation of *C. vulgaris* in BG11-medium and emphasized the particle/algae ratio as a primary criterion for efficient harvesting. However, it is important to note that they employed nanoparticles, whereas natural microparticles with significantly lower specific surface areas were utilized in this study. In addition, natural MMPs obtained through milling may also exhibit less active surface sites compared to synthesized particles commonly used in the literature, which possess high specific surface areas and higher density of functional groups on the surface (Barros et al., 2015; Safarik et al., 2009). The comparison indicates the involvement of additional factors in the interaction mechanism between particles and algae that potentially prevail over the influence of the particle surface, especially when using MMPs instead of nanoparticles.

Several studies suggest that the interaction between particles and algae could be an electrostatically driven process, as the separation was more efficient under acidic conditions (Wang et al., 2015). While most microalgae exhibit a negative zeta potential above pH 3 (Danquah et al., 2009), magnetite is a natural iron oxide with an isoelectric point in the neutral range (Schobesberger et al., 2021). Therefore, increasing the ionic strength of the suspension should affect the electrostatically driven process due to double-layer compression and the shielding of electrostatic forces at the interface. However, Fig. 1 (b) shows a negligible influence of the ionic strength on the separation. Consequently, electrostatic interactions may play a lesser role in the interaction when natural MMPs are employed.

The favorable interaction between particles and microalgae in this study may be attributed to several factors. Firstly, the composition of the microalgae cell surface, including promoting components like bridging proteins or locally charged surface groups, could facilitate the interaction (Cerff et al., 2012). Additionally, extracellular polymeric substances (EPS), present in the culture medium due to favorable cultivation conditions and the growth phase during harvest, may support the particle/algae interaction. Danquah et al. (2009) observed improved intercellular interactions and cell agglomeration during the stationary growth phase, leading to enhanced separation efficiency.

Furthermore, some nutrient salts of the culture medium, such as bivalent and trivalent ions, can improve the particle/algae interaction, particularly at higher pH values (Sukenik et al., 1984; Vandamme et al., 2012; Schobesberger et al., 2021). Interestingly, the results show that increasing the pH of the suspension leads to higher separation efficiencies, contrary to the expected behavior based on the zeta potential of both algae and particles.

Considering the subsequent dewatering steps of the microalgae and process costs, it is desirable to minimize the amount of particles used. Therefore, following experiments were conducted using particle/algae ratios of $1\,g_{MMP}\,g_{algae}^{-1}$ to further explore potential factors that could improve the interaction and enhance the separation efficiency. Additionally, investigating the increased influence of other factors under low particle concentration will aid in gaining a better understanding of the mechanism leading to the formation of particle/algae agglomerates.

3.2. *Influence of calcium and phosphate*

Separation experiments were performed in a model medium to restrict interaction phenomena to the influences of natural MMPs and microalgae, aiming to identify the underlying mechanisms. The model medium contained 10 mM NaCl to ensure the appropriate osmotic pressure. Fig. 2 shows the achieved separation efficiencies for the control samples (only MMPs and microalgae) and suspensions that additionally contained certain nutrient salts. The comparable removal efficiencies obtained for the control samples of around 50% indicate that pH has little effect on the interaction within the studied range. Given the expected decrease in zeta potential for both the algae and the MMP as

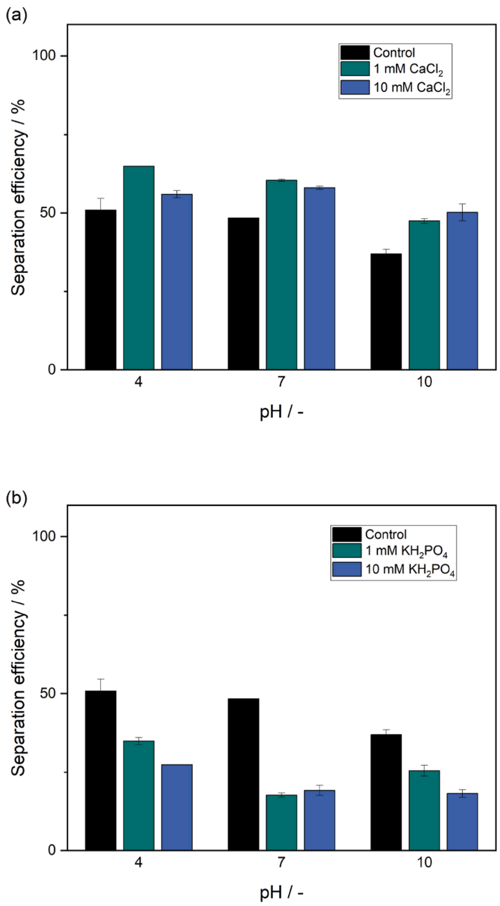

Fig. 2. Influence of different concentrations of divalent calcium ions (a) and free phosphate ions (b) on the separation efficiency of 1 g L^{-1} *C. vulgaris* with 1 g_{MMP} g_{algae}^{-1} MMPs in model medium containing 10 mM NaCl at acidic, moderate, and basic pH conditions.

pH increases, the results imply the presence of an adequate number of locally positively charged groups on the algal surface. These charged groups may sufficiently facilitate electrostatic interactions and enhance the overall interaction between algae and magnetite particles to a certain extent. A decrease in efficiency was only observed at pH 10, where both MMPs and microalgae exhibit clear negative zeta potentials, leading to stronger repulsion. The discrepancy with the behavior observed in BG11-medium suggests that the previously increased performance at basic pH may be due to the presence of certain nutrient salts, such as calcium or magnesium. Furthermore, the comparable results in the model medium and BG11-medium at low pH values indicate that separation in the acidic pH range is hardly influenced by external factors, such as medium composition. Therefore, efficient separation under these conditions can be challenging. For successful harvesting in this range, surface functionalization of the microparticles may be necessary. Unlike the natural iron ores, nanoparticles often yield higher separation efficiencies at acidic pH values, signifying a higher influence of electrostatic attraction forces (Hu et al., 2013; Prochazkova et al., 2013; Xu et al., 2011).

Contrary to previous expectations, the experiments on particle/algae interaction reveal that the addition of divalent ions, such as calcium, has a negligible influence on the separation efficiency within the pH range of 4 to 10 (see Fig. 2). The addition of 1 or 10 mM calcium chloride results in no clear trend and only slightly increased separation efficiencies. However, Fig. 2 (b) shows that free phosphate ions can negatively affect and inhibit particle/algae interaction. Prochazkova et al. (2013) reported significantly increased separation efficiencies for harvesting *C. vulgaris* with magnetic iron oxide microparticles in a mineral medium lacking phosphate, as well as a strong phosphate dependence of the zeta potential of the particles at pH 6.5. Competitive adsorption of dissolved phosphate and the microalgae at the binding sites of the MMP is suspected, as loading experiments in water show a high affinity of phosphate for the iron oxides. Daou et al. (2007) showed that phosphate interacts with positively charged groups and hydroxyl groups on the surface of magnetite, with affinity decreasing at higher pH values. Nevertheless, the experiments display an inhibition of the particle/algae interaction even at basic pH, implying additional interactions. Zeta potential measurements further reveal that free phosphate ions induce a slight negative shift in the surface charge of the algae, which leads to enhanced dispersion stability (see Fig. 3 (c)). The negative influence of phosphate is particularly relevant because some cultivation media deviate from the optimal N/P ratio and overdose phosphate to use it as a buffer. This should particularly be considered in wastewater treatment by microalgae cultivation, where the algae are used to reduce high phosphate levels (Chu et al., 2013).

Intriguingly, the combined addition of both salts cancels the negative influence of phosphate and leads to a significantly improved magnetic separation of *C. vulgaris* (see Fig. 3 (a)). While lower influences are observed in the acidic range, significantly increased harvesting efficiencies > 98% can be achieved depending on the salt concentrations above pH 7. This phenomenon is based on the pH-induced decrease in solubility of calcium phosphates, which precipitate with increasing pH (Sukenik et al., 1984; Beuckels et al., 2013). Precipitation of the resulting salts occurs predominantly by heterogeneous nucleation on the particle and cell surfaces, with hydroxyapatite (HAP) being the most stable calcium phosphate compound in this pH range. However, several other compounds can precipitate as precursors to HAP, such as dicalcium phosphate dihydrate, $Ca_3(PO_4)_2$, or amorphous calcium phosphate (Van Der Houwen and Valsami-Jones, 2001).

Fig. 3 (c) depicts the consistently negative zeta potential of approximately 20 mV of *C. vulgaris* within the pH range of 4 - 10, both in the model medium and with phosphate addition. However, the addition of calcium phosphates leads to an increasing reduction of the algal net surface charge. At pH 10, the zeta potentials were slightly inconclusive due to the starting flocculation and sedimentation of the algae during measurement, despite the suspension being diluted. Nonetheless, the measured values align with data presented by Beuckels et al. (2013). The precipitation of calcium phosphates leads to charge neutralization of the algal/particle suspension due to locally positive surface charges, thereby reducing repulsion and facilitating flocculation/agglomeration phenomena. The resulting MMP/microalgae composites after flocculation were illustrated with optical and fluorescence microscopy (see supplementary material). pH-induced flocculation of algal cells is accompanied by entrapment of magnetic ores, facilitating magnetic separation of the particle/cell composites in a magnetic field (see supplementary material). However, the larger sludge volume associated with higher flocculant dosages has to be considered, underscoring the importance of determining the optimal flocculant dosage.

ICP-OES measurements of calcium and phosphorus concentration in the supernatant after separation support the hypothesis of pH-induced flocculation (Fig. 3 (b) and (d)). At pH 4, ion concentrations remain unchanged, while at pH 7 and 10, high salt dosage (10 mM Ca and PO_4) corresponds to a notable reduction in ion concentrations. In addition, the slight decrease in calcium concentration and the increase in phosphorus concentration at pH 10 can be attributed to several phenomena. Increasing pH causes higher supersaturation, leading to increased precipitation. Additionally, the affinity of phosphate for MMPs diminishes as pH rises. The results further indicate that good separation efficiencies above 90% are only attained when precipitation of calcium phosphate species occurs. In the absence of precipitation, samples with low salt dosage yield a separation performance comparable to the control sample (pH 4 and 7), while efficiencies above 97% are achieved at pH 10 (see supplementary material).

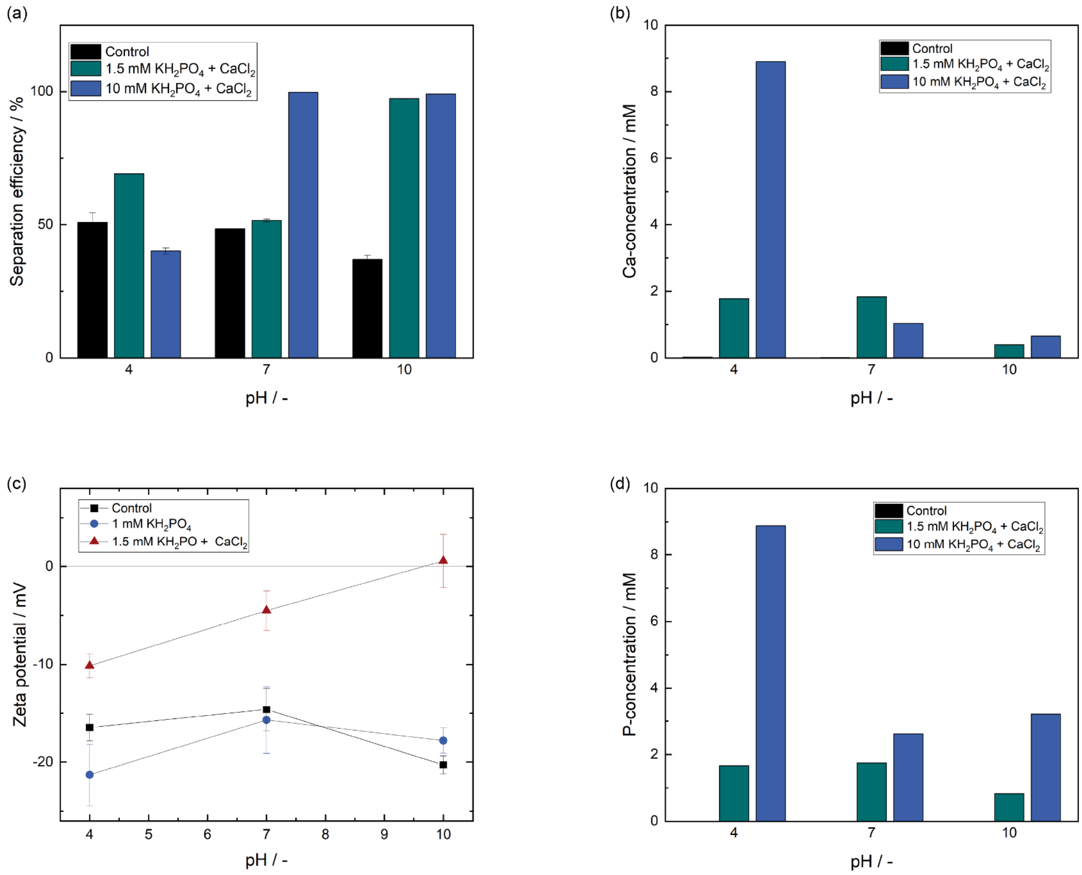

Fig. 3. Influence of pH-shift induced calcium phosphate precipitation on magnetic separation of $1\,g\,L^{-1}$ *C. vulgaris* with $1\,g_{MMP}\,g_{algae}^{-1}$ MMPs in model medium with 10 mM NaCl. (a) Separation efficiency dependence on pH and calcium phosphate concentration in the medium. ICP-OES measurements of calcium (b) and phosphorus (d) remaining in the supernatant after magnetic separation. (c) Influence of phosphate and calcium phosphate on the zeta potential of a diluted microalgae culture.

The critical pH for precipitation correlates with the salt concentration and medium composition. Previous studies have shown that calcium phosphate precipitation can occur due to a significant decrease in solubility above pH 7 (Malkaj et al., 2005; Bell et al., 1973). Van Der Houwen and Valsami-Jones (2001) observed spontaneous precipitation at 25 °C, pH 7, an ionic strength of 0.1 M, and a near stoichiometric calcium/phosphate ratio for hydroxyapatite. Yong-hui et al. (2006) further suggests adding calcium in more abundance as inhibiting substances could affect precipitation. A higher Ca/P ratio can also shift precipitation to lower pH values or salt concentrations and reduces potential negative interactions of free phosphate ions. Based on the results and cost considerations, the optimal pH range for pH-induced calcium phosphate precipitation should be between pH 8 and 9. This approach enhances process robustness, even at lower flocculant doses. Moreover, under suitable culture conditions, algal metabolism can naturally achieve the desired pH range without the need for bases.

The presented approach using pH-induced calcium phosphate precipitation is versatile across various algal species and particles, enabling broad applicability for harvesting microalgae with minimal adjustments. Moreover, when combined with the low-cost natural magnetite particles, it offers a cost-effective and energy-efficient means of algae separation using appropriate high-throughput magnetic separators. Therefore, further studies should focus on developing tailored magnetic separation systems to unlock the full potential of this innovative method. These systems should ensure selective and efficient harvesting of diverse microalgae while avoiding excessive dilution of the particle/algae sludge.

3.3. *Influence of algogenic organic matter*

Investigating the influence of organic compounds on the combined MMP-mediated flocculation approach is essential to establish a robust and conservatively estimated process. Thus, Fig. 4 (a) illustrates the impact of a culture solution, which exhibits high TOC loads after cultivation, on pH-shift induced calcium phosphate precipitation and the efficiency of magnetic harvesting of $2.1\,g\,L^{-1}$ *C. vulgaris*. Compared to the separation in the model medium, a strong inhibitory effect on the particle/algal interaction occurs, with the control samples yielding a separation efficiency below 25% across the entire investigated pH range. At low flocculant dosage, calcium phosphate precipitation is also strongly inhibited since there is no visible flocculation and only a slight increase in efficiency. ICP-OES measurements show no change in calcium concentration in the supernatant of the control sample (see Fig. 4 (b) and (c)). Measurements of the algae culture further indicate a complete consumption of phosphate during the cultivation, as concentrations are below the detection limit. At low flocculant dosage, a slight decrease in phosphorus concentration occurs with increasing pH, which is even more prominent for calcium. However, these changes are significantly lower than those observed in the model medium.

The inhibitory effect of the culture solution can be attributed to algogenic organic matter (AOM), which microalgae secrete into the medium as a stress response during cultivation. AOM consists of a mixture of different proteins and polysaccharides. Vandamme et al. (2016) suspected that high molecular weight anionic polysaccharides with properties similar to alginic acid or alginate are likely responsible for this inhibitory effect. These polysaccharides have a linear polymer structure comprising guluronate and mannuronate, with carbonyl and

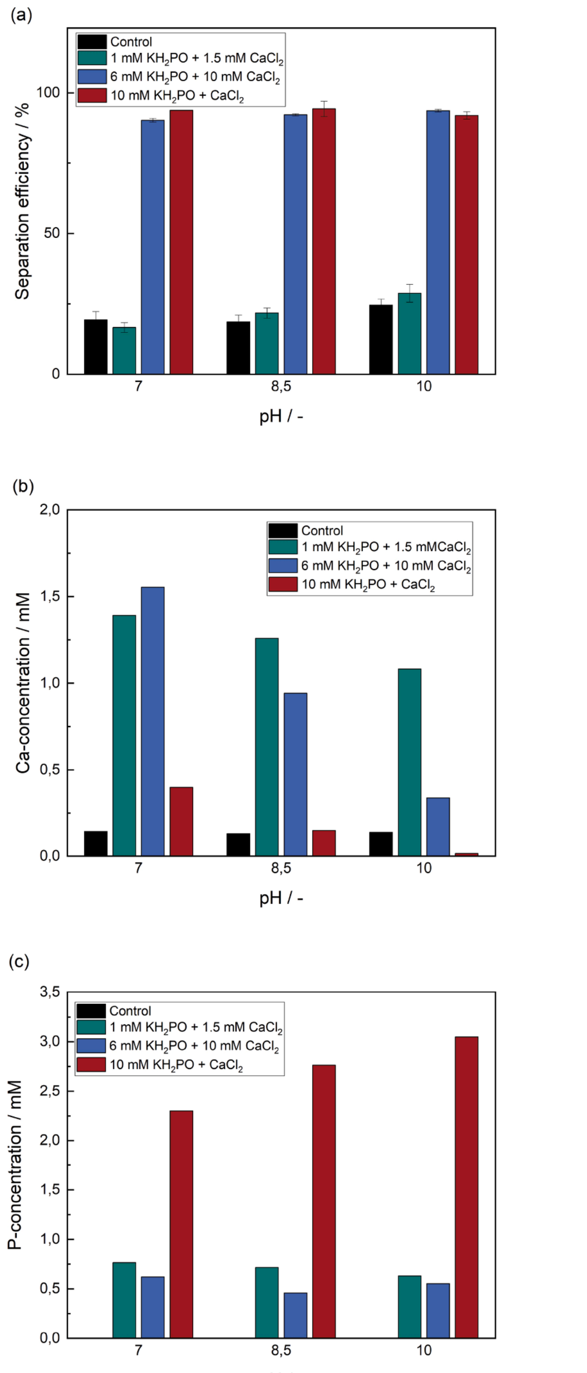

Fig. 4. (a) Influence of original culture medium containing high amounts of algogenic organic matter (AOM) on pH-shift induced calcium phosphate precipitation and magnetic separation of $2{,}1\,g\,L^{-1}$ *C. vulgaris* with $1\,g_{MMP}\,g_{algae}^{-1}$ MMPs. pH was varied between 7 and 10 since salt precipitation and flocculation in the model medium were only observed above pH 7. ICP-OES measurements of calcium (b) and phosphorus (c) remaining in the supernatant.

carboxylic acids as the main functional groups. The cell surface composition and AOM secretion further depend on the growth phase of the algae. A study by Rashid et al. (2019) reported that AOM loading and its inhibitory effect on flocculation increases with a more prolonged growth phase. Furthermore, the nature of AOM varies with the growth phase, with loose secretion into the medium during the exponential growth phase and cell-bound form during the stationary growth phase.

TOC measurements of the culture supernatant revealed a total organic carbon concentration of $39\,mg\,L^{-1}$. Several studies have reported that excess AOM increases repulsion and decreases flocculation efficiency (Beuckels et al., 2013; Vandamme et al., 2012). Besides binding to the active sites of the MMP, AOM affects the particle/algae interaction by shielding the positively charged groups on the surface of microalgae or MMPs and stabilizing the dispersion. In addition, organic compounds can have an inhibitory impact on the precipitation of calcium phosphates (Yong-hui et al., 2006; Van Der Houwen and Valsami-Jones, 2001). AOM influences pH-induced precipitation by complexation of calcium or binding to the active growth sites of precipitates. Malkaj et al. (2005) reported a very high affinity of sodium alginate for HAP, resulting in the inhibition of HAP crystal growth at low concentrations and reducing crystal growth rates by 42-86% at inhibitor concentrations of 2.1×10^{-4}-12.6×10^{-4} mM. The calcium chelation decreases precipitation efficiency and increases calcium consumption. The interaction between AOM and calcium may also shift precipitation to higher pH values and reduce the conversion rate of calcium phosphate to thermodynamically more stable compounds, such as hydroxyapatite (Alvarez et al., 2004). Furthermore, it is important to note that high microalgae concentrations, as utilized in this study, can adversely impact the combined flocculation process, particularly when using low flocculant quantities. The flocculation mechanism changes depending on the concentration of the precipitating salts (García-Pérez et al., 2014). Significant precipitation can occur at high salt concentrations, leading to flocculation through a sweeping mechanism and reduced dependence of the flocculant dosage on the biomass concentration. On the other hand, charge neutralization predominates at low salt concentrations, causing the minimum flocculant dosage or pH to increase with higher biomass concentration.

Due to the inhibition, successful flocculation requires higher salt concentrations or elevated pH values. Fig. 4 (a) shows that increased calcium concentrations of 10 mM enable harvesting efficiencies above 90% at moderate pH values. Interestingly, ICP-OES measurements of the supernatant from experiments with stoichiometric salt addition for hydroxyapatite (HAP) reveal a significant decrease in calcium as pH increases, while phosphorus concentrations remain constant (see Fig. 4 (b) and (c)). Thus, the increased calcium consumption is not due to precipitation but rather interactions with other components, such as AOM. Furthermore, the results suggest that the interactions between AOM and calcium could be pH-dependent and become more pronounced with increasing pH. Despite successful flocculation, the remaining phosphate (0.5 mM) and relatively high calcium concentration (above 1 mM) indicate an inhibition of the precipitate growth below a specific concentration threshold. In contrast, adding an equal calcium/phosphate ratio yields a complete precipitation of calcium at high pH. The increase in phosphorus concentration with increasing pH observed in this case can be attributed to two factors. Firstly, the interaction of calcium with AOM intensifies, causing a precipitation shift toward hydroxyapatite, as evidenced by the rising Ca/P ratio in the ICP-OES measurements (from 1.42 at pH 7 to 1.63 at pH 10). Secondly, the affinity of phosphate for the magnetite particles decreases.

The results suggest that an excess of calcium should be added to compensate for the higher consumption due to the AOM interaction and to facilitate precipitation more effectively. As phosphate is significantly more expensive, minimal phosphate amounts are preferred when determining the flocculant dosage. Furthermore, it is desirable to minimize the dosage as excess flocculants and high pH levels increase floc size and may impede subsequent dewatering steps. TOC measurements prior to harvesting are suitable to estimate the AOM load in the culture solution and thus determine an appropriate salt dosage. Considering the strong inhibitory effect of AOM on magnetic separation and classical flocculation processes, optimizing and actively controlling cultivation conditions are essential to minimize stress effects on the algae. The cultivation time should be determined based on the desired product and economic efficiency. While harvesting in the exponential growth phase is a more cost-effective approach due to the lower salt requirement for the combined flocculation process, the stationary growth phase produces a considerable amount of the lipids desired for many applications. Additionally, it is advisable to process the algae immediately after cultivation, as prolonged storage leads to an increased AOM load.

3.4. *Magnetite microparticle detachment*

The reusability and regeneration of the MMPs are critical to the design of a sustainable algae harvesting process. Hence, separation experiments were conducted at pH 8.5, while subsequently shifting to pH 5 to investigate the detachment. Fig. 5 (a) shows the achieved separation efficiencies in AOM-containing culture solutions for different flocculant doses as well as the results of the detachment experiments. In addition, different amounts of NaCl were added to the suspensions with high flocculant dosage to investigate the influence of ionic strength on separation and detachment. At pH 8.5, medium-dose solutions achieve removal efficiencies of approximately 75%, while high salt dosage leads to efficiencies ranging from 83 to 94%, depending on the set ionic strength. However, the increase in ionic strength does not show a clear trend. Sodium ions can substitute calcium at the surface of precipitated salts and thereby enhance flocculation by replacing calcium ions consumed in AOM interaction (Bell et al., 1973). The larger sludge volume in this case also indicates increased flocculation (see Fig. 5 (b)). However, further increasing the ionic strength tended to decrease the flocculation efficiency. Since ionic strength has little effect on the interaction between particles and algae, this could indicate a weakening of pH-induced flocculation at high ionic strengths due to the shielding of the locally charged salts. Whereas, slight acidification of the suspension to pH 5 dissolves the precipitated salts regardless of the ionic strength and reduces the sludge volume to the initial particle volume V_0.

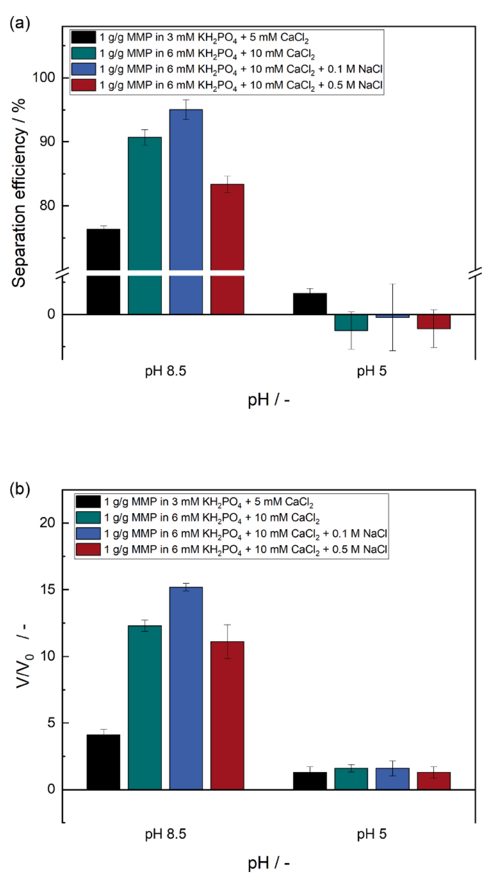

Fig. 5. Particle detachment experiments after separation of 2.1 g L^{-1} *C. vulgaris* with 1 g_{MMP} g_{algae}^{-1} MMPs in culture medium containing high AOM loads. Influence of different flocculation dosages and ionic strength on the separation efficiency (a) and sludge volume increase (b) at pH 8.5 and 5. Acidification after separation dissolves the precipitated salts and reduces the sludge volume V to the initial volume of the sedimented particles V_0.

The detachment studies highlight a significant advantage of the combined MMP-mediated and pH-induced salt precipitation process for magnetic microalgae separation. Mild acidification of the suspension achieves complete and fast detachment of the microalgae, as shown by the negligible separation efficiencies of algae in Fig. 5 (a). In consequence, the MMPs can be selectively separated with a magnet and reused. In addition, acidification enables the recovery of added calcium phosphates, enhancing process economics and sustainability. The low particle/algae interaction in the acidic pH range results from inhibition by AOM and reduced impact of electrostatic interactions when employing natural MMPs. In addition, the particles exhibit magnetic remanence after separation, as shown in Fig. 1 (c). The resulting magnetic attraction causes agglomeration of the MMPs, leading to a reduced available particle surface for algal interaction. In general, Fig. 5 shows that algae harvesting using pH-induced flocculation is reversible and thus enables efficient separation of the algae with simultaneous easy recycling of the particles. Following detachment, near-complete recoveries close to 100% were achieved by magnetic separation of the MMPs. Even after five cycles of algae separation, detachment, and recovery, particle recovery remained consistently above 98% of the initial particle quantity. Furthermore, initial examinations of the reusability of the natural MMPs were performed to assess the performance of the reused particles. Across five cycles, the MMPs exhibited a decrease in harvesting efficiency ranging from 5% to 10% compared to the original performance. This decline may be attributed to various factors, including the potential adverse influence of lingering algae and AOM attachment. Additionally, magnetic remanence-induced agglomeration of the MMPs might lead to a reduced available surface area for interaction with the algae.

However, Fig. 5 (b) also reveals that pH-induced flocculation can cause an undesired increase in sludge volume. The particle/algae sludge formed after separation contains substantial volumes of bound water due to flocculation. Overdosing flocculants or using significantly higher pH values than required leads to massive precipitation and larger flocs (García-Pérez et al., 2014). As a result, solutions with high salt dosage have 3-4 times larger sludge volumes than those with medium salt dosage. Therefore, a compromise between good separation efficiency and low water content of the resulting sludge must be found. Thus, optimal flocculation dosage selection becomes critical for designing an appropriate process point.

4. Conclusion

pH-induced calcium phosphate precipitation enhances MMP/microalgae interaction and enables harvesting efficiencies of 98% at moderate pH and low particle dosage. This approach attenuates the inhibitory effect of AOM while facilitating fast and reversible MMP recycling through mild acidification. The combined approach of pH-induced flocculation with MMPs enables generalized and energy-efficient microalgae harvesting by high-throughput magnetic separators. Further studies should focus on developing tailored and automated magnetic separation systems to unlock the full potential of this process, advancing the utilization of microalgae as a sustainable bioresource.

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CRediT authorship contribution statement

Sefkan Kendir: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Matthias Franzreb:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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