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Influence of magnesium concentration, biomass concentration and pH on flocculation of *Chlorella vulgaris*

J. Saúl García-Pérez[a], Annelies Beuckels[b], Dries Vandamme[b1], Orily Depraetere[b], Imogen Foubert[b], Roberto Parra[a], Koenraad Muylaert[b]

[a] Water Center for Latin America and Caribbean, Tecnologico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, 64849 Monterrey, Nuevo Leon, Mexico.

[b] Laboratory Aquatic Biology, KU Leuven Kulak, E. Sabbelaan 53, Kortrijk, Belgium

[1]Corresponding author:
Email: dries.vandamme@kuleuven-kulak.be
Tel: +32 56 246041
Fax: +32 56 246999

Abstract

Autoflocculation is a promising low-cost method for harvesting microalgae for bulk biomass production or wastewater treatment. Autoflocculation can be caused by precipitation of calcium or magnesium at high pH. In this study, we investigated the interactive effects of pH, magnesium concentration and microalgal biomass concentration on flocculation of *Chlorella vulgaris* by magnesium hydroxide. The minimum pH for inducing flocculation was lower when magnesium concentration in the medium is higher. A higher pH and/or higher magnesium concentration are required for flocculation when microalgal biomass concentration is increased. The sludge volume formed during flocculation is highly variable and is influenced mainly by the amount of magnesium hydroxide that precipitates during flocculation. The sludge volume increases with pH and with magnesium concentration in the medium. There is an optimal pH where flocculation efficiency is maximized (> 95%) and sludge volume is minimal (1 - 2% of culture volume). Increasing the pH slightly above this optimum results either in an increase in sludge volume and/or a decrease in flocculation efficiency. We propose that autoflocculation by magnesium hydroxide can be more easily controlled by the dosage of base rather than by targeting a specific pH level.

Keywords: flocculation - brucite – magnesium hydroxide - microalgae – harvesting – biomass- coagulation

1. Introduction

Microalgae are a promising new source of biomass for production of food, feed, fuel and bulk chemicals or for treatment of wastewater [1,2]. Although microalgae are already being produced for high-value products, the cost of production should be reduced by at least an order of magnitude to become a commodity crop [3]. A major challenge in realizing large-scale and low-cost production of microalgae is developing a harvesting technology that can process large volumes of water at a minimal cost [4,5].

Flocculation holds a lot of potential as a low-cost method for microalgae harvesting [6,7]. An interesting approach for flocculating microalgae is autoflocculation induced by high pH [5]. Autoflocculation at high pH is the result of precipitation of calcium and magnesium salts. Flocculation can be induced by precipitation of calcium phosphates at pH 8-9, but relatively high phosphate concentrations are required for this process to occur [8,9]. In medium with low phosphate concentrations, flocculation can be induced by magnesium hydroxide precipitation, further referred to as magnesium flocculation. Magnesium hydroxide precipitates at a pH above 10.5 and these precipitates are positively charged up to pH 11.5. These positively charged magnesium hydroxide precipitates can interact with the negatively charged surface of microalgal cells and induce flocculation [10,11]. Because background concentrations of magnesium in most waters are high enough for magnesium flocculation to occur, the only cost involved is the cost of the base used to increase pH [12].

Most studies that explored the potential of magnesium flocculation for harvesting microalgae focused on the flocculation efficiency. A high flocculation efficiency indicates that most algal cells in the medium have flocculated and settled. The flocculation efficiency is thus a measure of the proportion of the biomass that can be harvested by the flocculation method. Studies dealing with magnesium flocculation have documented how the flocculation efficiency is influenced by pH or the magnesium concentration in the medium and how the minimum pH required to achieve a high flocculation efficiency increases with biomass concentration [11,12].

Although flocculation efficiency is an important parameter when evaluating the technical feasibility of magnesium flocculation, other parameters are equally important. When flocculation is used for harvesting microalgae, it is part of a two-stage process in which flocculation is used to remove the bulk of the water, while a mechanical method is used for subsequent dewatering [13,14]. To minimize the cost for mechanical dewatering, it is important that the volume of sludge produced by flocculation is as small as possible. Some studies have reported that the sludge volume may be highly variable in the case of magnesium flocculation. In a study on magnesium flocculation of *Phaeodactylum tricornutum*, it was observed that the sludge volume became very large at high pH [15]. Experiments with autoflocculation of *Chlorella vulgaris* also noted an increase in sludge volume in magnesium flocculation at high pH levels [10]. It is not fully clear, however, what causes these differences in sludge volume.

The goal of this study was to understand how both the flocculation efficiency and sludge volume are influenced by pH and by the magnesium concentration in the medium. This information is important to define optimal conditions for magnesium flocculation of microalgae that maximize the flocculation efficiency and minimize the sludge volume. Based on our results, we propose an approach for implementing magnesium flocculation of microalgae that avoids an excessive accumulation of sludge while ensuring a high flocculation efficiency.

2. Material and methods

2.1 Cultivation of Chlorella vulgaris

The strain *Chlorella vulgaris* 211-11b (SAG, Germany) was used as a model species for studying magnesium flocculation. *Chlorella* has previously been used as a model species in autoflocculation studies (e.g. [10,12]). *Chlorella* was cultured in Wright’s Cryptophyte (WC) medium in 30 L plexiglass bubble column photobioreactors (20 cm diameter). The reactors were aerated with 0.2 µm filtered air (5 L min^{-1}) and pH was maintained at 8.5 through pH-controlled addition of carbon dioxide to the air flow. The culture was irradiated from two sides with daylight fluorescent tubes, giving a light intensity of 60 µEinst m^{-2} s^{-1} at the surface of the reactor. Algal biomass was monitored by measuring absorbance at 750 nm [16]. Absorbance measurements were calibrated against dry weight. Dry weight was determined gravimetrically on pre-weighed GF/F glass fiber filters [16]. 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}, which is a typical biomass concentration encountered in open pond production systems. Initial biomass concentration was typically 0.01-0.02 g L^{-1}.

2.2 Flocculation experiments

To study the influence of magnesium concentration on flocculation efficiency and sludge volume, *Chlorella* cells were harvested by centrifugation and resuspended in fresh medium. Previous experiments had shown that centrifugation and resuspension in fresh medium has no significant influence on flocculation [9]. This fresh medium was identical to the original Wright's Cryptophyte medium but lacked calcium and magnesium. No calcium was added to avoid precipitation of calcium phosphate or calcium carbonate, which could theoretically also cause flocculation. Magnesium was added to the medium in the desired level by addition of magnesium sulphate. The pH of the medium was adjusted by addition of 0.5 M HCl or 0.5 M NaOH to initiate precipitation of magnesium hydroxide and to induce magnesium flocculation. To study the influence of biomass concentration on magnesium flocculation, *Chlorella* cells were resuspended in fresh medium at different concentrations (0.1, 0.2, 0.4, 0.8 or 1.6 g dry weight L^{-1}). All experiments were carried out in triplicate.

The experiments to study the influence of biomass concentration, pH and magnesium concentration on flocculation efficiency were carried out in jars containing 100 mL of broth and mixing was achieved by a magnetic stirrer. Evaluation of sludge volume was performed in 100 mL measuring cylinders. The experiments to study the influence of pH and magnesium concentration on sludge volume were performed using 1 L broth and mixing was done using an overhead mixer. pH was adjusted during 10 min of intensive mixing at 1000 rpm, followed by 20 min of gentle mixing at 250 rpm. The suspensions were then allowed to settle for 30 minutes. Evaluation of the sludge volume was performed in 1 L Imhoff cones. The flocculation efficiency was estimated from changes in the optical density (measured at 750 nm) prior to pH adjustment (OD_i) and after settling (OD_f). The flocculation efficiency (η_a), or the percentage of microalgal biomass removed from suspension, was calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} . 100$$

2.3 Zeta potential measurements

To confirm that flocculation was caused by charge neutralisation, we measured changes in the zeta potential of the cells during flocculation. Zeta potential of cell suspensions were measured during stepwise increase of medium pH using a Malvern Zetasizer Nano. Zeta potential measurements were carried out in a control medium lacking magnesium and a medium with magnesium (0.75 mM).

2.4 Measurements of magnesium concentration

To quantify the amount of magnesium hydroxide precipitates formed in some experiments, we compared concentrations of magnesium in the medium before and after flocculation. Magnesium concentrations in the medium were measured using ICP-OES (Perkin Elmer, Optima 3300 DV).

3. Results and discussion

3.1 Influence of magnesium concentration and pH on the flocculation efficiency

We investigated the effects of free magnesium concentration and pH on the flocculation efficiency in medium with a *Chlorella* biomass concentration of 0.25 g L^{-1} (Figure 1). At a very low magnesium concentration (addition of 0.01 and 0.03 mM magnesium sulphate), no flocculation occurred even when pH was increased to 12. At a magnesium concentration of 0.1 mM, flocculation occurred only at the highest pH levels tested (11.5 and 12), and even then the flocculation efficiency remained below 90%. At higher magnesium concentrations (0.3 and 1 mM), flocculation occurred already at a pH of 10.5 and the flocculation efficiency was always higher than 90%. Our results show that the minimum pH to induce flocculation decreases when magnesium concentration in the medium increases. Magnesium flocculation is the result of precipitation of magnesium hydroxide. Precipitation of magnesium hydroxide is a function of the product of free magnesium and hydroxide concentrations, the latter increasing in proportion to pH. Thus, when magnesium concentration is high, the amount of hydroxide anions required to initiate precipitation is low and flocculation will occur at a low pH. When magnesium concentration is low, higher hydroxide concentrations and thus a higher pH are required for magnesium hydroxide precipitation and flocculation to occur.

Magnesium flocculation is assumed to follow a similar mechanism as flocculation by metal salts such as alum or ferric chloride, where flocculation is also caused by metal hydroxide precipitates [10,17]. The minimum metal dose for inducing flocculation with metal salts is in the same order of magnitude as observed in this study: 0.3 to 0.7 mM for both ferric chloride flocculation [18,19] and aluminum chloride flocculation [20,21]. This is comparable to observations of magnesium flocculation in a previous study, where 0.25-0.6 mM of magnesium was required to flocculate *Chlorella* and *Scenedesmus* [22]. In this study as well as in another study of magnesium flocculation of *Chlorella* [12], the minimum concentration of magnesium was slightly lower than reported in previous studies 0.15 mM. This lower minimum concentration may be due to the fact that our experiments were carried out with microalgal cells resuspended in fresh medium that did not contain dissolved organic matter excreted by microalgae. This excreted algal organic matter is known to interfere with flocculation and to result in an increased flocculant dose [23,24].

3.2 Zeta potential measurements

To understand the underlying mechanism of magnesium flocculation, we carried out measurements of zeta potential of *Chlorella* cells in medium with and without magnesium. In the absence of magnesium, the zeta potential of *Chlorella* cells remained negative and constant over a pH range of 9 to 12 (Figure 2). When the medium contained 0.75 mM magnesium, the zeta potential approached 0 in the pH range 10.75 to 11. Above pH 11.5, the zeta potential decreased again.

Magnesium hydroxide precipitates have a point of zero charge of about 11.5 [25]. Therefore, the surface charge of magnesium hydroxide is positive below pH 11.5. Our measurements of zeta potential suggest that these positively charged precipitates attach to the negatively charged microalgal cells and neutralize the surface charge. The observed decrease in zeta potential above pH 11.5 can be explained by the fact that the surface charge of magnesium hydroxide precipitates changes from positive to negative at this pH. Our observations are in agreement with Wu et al. [11], who also observed an increase in zeta potential at high pH levels during magnesium flocculation. This neutralization of the surface charge may cause flocculation. Most likely, sweeping flocculation also contributes to the flocculation mechanism, especially at high magnesium concentrations and/or high pH levels, when massive amounts of magnesium hydroxide are formed [17].

3.3 Influence of biomass concentration on the minimum pH and magnesium concentration required for flocculation

If flocculation is primarily due to charge neutralization, then the amount of magnesium hydroxide required to induce flocculation should increase with increasing biomass concentration. We therefore prepared suspensions of *Chlorella* cells with different biomass concentrations in medium with either a low (0.5 mM) or a high (1.5 mM) magnesium concentration and adjusted the pH to different levels. When biomass concentration was low (0.1 or 0.2 g L^{-1}), flocculation could be induced at pH of 10.5 or higher, irrespective of the magnesium concentration (Figure 3). When biomass concentration was 0.4 or 0.8 g L^{-1}, flocculation occurred at pH 10.5 when magnesium concentration was high while a higher pH was required when magnesium concentration was low. At the highest biomass concentration of 1.6 g L^{-1}, no complete flocculation could be induced in the medium with low magnesium concentration while flocculation was still possible at pH 11 and 12 in the medium with higher magnesium concentration.

These observations indeed suggest that the amount of magnesium hydroxide required for flocculation increases with increasing biomass concentration. In a previous study on magnesium flocculation, Wu et al. [11] noted that the minimum pH to induce flocculation increased when biomass concentrations were higher. Schlesinger et al. [5], on the contrary, reported that flocculation at high pH was almost independent of biomass concentration. These differences may be due to differences in the magnesium concentration in the medium. When magnesium concentration in the medium is high, massive precipitation of magnesium can occur and this causes flocculation by a sweeping mechanism. When sweeping flocculation occurs, the flocculant dose tends to be independent of biomass concentration [19]. When magnesium concentration is low, charge neutralization is most likely more important than sweeping flocculation and the minimum dose of magnesium will increase with increasing microalgal biomass concentration.

3.4 Influence of magnesium concentration and pH on the sludge volume

In previous studies on magnesium flocculation it was noted that the sludge volume is highly variable [10,15]. To better understand what causes variation in sludge volume, we investigated the effects of magnesium concentration and pH on the algal sludge volume (Table 1). Experiments were carried out at a biomass concentration of 0.25 g L^{-1} *Chorella* biomass. Magnesium concentrations used in this experiment were relatively high (5, 10 and 15 mM) in order to achieve a high flocculation efficiency at all pH levels tested. Indeed, the flocculation efficiency was always 95% or higher, except in pH 10.5 treatments with 5 and 10 mM magnesium, where the flocculation efficiency was close to 90%.

From Table 1, it can be observed that the algal sludge volume increased with both increasing pH and increasing magnesium concentration. When magnesium concentration in the medium was 5 or 10 mM, the algal sludge volume was below 30 mL (on a total volume of 1 L) when pH was only raised to 10.5. But the sludge volume increased to between 100 and 300 mL when pH was raised to 11 or 12. When magnesium concentration in the medium was 15 mM, the algal sludge volume varied from 120 mL at pH 10.5 to more than 400 mL when pH was increased to 11 or 12. Reproducibility was only tested at a single combination of pH and magnesium concentration but was relatively good (standard deviation 2.6% of mean).

We also measured the magnesium concentration that remained in the medium after flocculation (Table 1) to estimate the amount of magnesium hydroxide that had precipitated during flocculation. When pH was raised to 10.5, less than half of the magnesium disappeared from the medium. When pH was raised further to 11, more than 80% of the magnesium had disappeared from solution. When pH was raised to 12, almost no magnesium was left in solution. The amount of magnesium that had disappeared from solution during flocculation was strongly correlated with the algal sludge volume (Pearson correlation coefficient 0.95, $p < 0.001$, $n = 9$; Figure 4). This clearly demonstrates that the volume of microalgal sludge formed during magnesium flocculation is highly dependent on the quantity of magnesium hydroxide precipitated. To minimize the sludge volume, it is important that the precipitation of magnesium hydroxide does not exceed the minimum dose required to induce flocculation. Flocculation by magnesium hydroxide precipitation is also used in waste.water treatment in the lime softening treatment [26]. In lime softening, it has also been shown that the sludge volume is a function of the amount of magnesium hydroxide precipitate that is formed [27].

3.5 Optimizing flocculation using magnesium hydroxide

From the results presented above, it is clear that overdosing of base should be avoided to minimize the sludge volume, at least when concentrations of magnesium in the medium are high. Therefore, we carried out an experiment to determine the optimal dose for magnesium flocculation, which is the base dose that maximizes flocculation efficiency and minimizes the sludge volume. We carried out two experiments with very different magnesium concentrations, a low (0.15 mM) and one with high (7.5 mM) magnesium concentration, in order to compare magnesium flocculation in soft waters and waters with a very high magnesium concentration (e.g. very hard water or brackish water). In different beakers, we adjusted the pH to different levels ranging from 9.5 to 12.5, with steps of 0.25 pH units. *Chlorella* biomass concentration was the same in both experiments (0.25 g L^{-1}). The flocculation efficiency, sludge volume and sodium hydroxide dose required to adjust the pH were recorded (Figure 5).

In the experiment with low magnesium concentration, no flocculation occurred up to pH 10.5. At pH 10.75, flocculation efficiency increased abruptly up to 94%. At this pH level, the sludge volume was 1.5 mL (on a total volume of 100 mL), which corresponds to a concentration factor of about 50. A further increase in pH increased the flocculation efficiency only slightly but resulted in about a doubling of the sludge volume. When pH was increased to 12 or higher, the flocculation efficiency and sludge volume both declined again. This decline can probably be ascribed to a reversal of the surface charge of the magnesium hydroxide precipitates at high pH. In this experiment, the optimal pH was thus 10.75. About 2 mM sodium hydroxide was added at this point in the experiment. Because the medium contained only 0.15 mM magnesium, only 0.3 mM of the 2 mM sodium hydroxide added could have been involved in precipitation of magnesium hydroxide, the remaining part being responsible for increasing free hydroxyl ions (causing the increase in pH) or being absorbed by buffers.

In the experiment with 7.5 mM magnesium in the medium, flocculation efficiency was 66% at pH 10 and increased to 95% at pH 10.25. At pH 10.25, the sludge volume was 1 mL (on a total volume of 100 mL), corresponding to a concentrating factor of about 100. When pH was increased further, the flocculation efficiency remained more or less constant but the sludge volume increased rapidly to a maximum value of 14 mL at pH 11.25, corresponding to a concentration factor of only 7. At pH 12, the flocculation efficiency and sludge volume declined slightly, but not as much as in the experiment with the low magnesium concentration. This may be due to the fact that flocculation was caused more by a sweeping mechanism than by charge neutralization. The optimal pH in this experiment was thus 10.25. Only 0.6 mM of sodium hydroxide was added at this point. This implies that maximum 0.3 of the 7.5 mM of magnesium had precipitated at pH 10.25.

These results suggests that, at the optimum pH, more or less the same amount of magnesium hydroxide had precipitated in both experiments. This is in agreement with our previous observation that a fixed amount of magnesium hydroxide is required to flocculate *Chlorella* cells at a biomass concentration of 0.2 – 0.3 g L^{-1}. The optimum pH in both experiments, however, was very different. At high magnesium concentration in the medium, flocculation occurs at a lower pH. When pH is increased above the optimum, the sludge volume increases and the concentration factor decreases, particularly when the magnesium concentration in the medium is high. This can relatively easily be avoided by limiting the amount of base that is added, as the dosage of base ultimately determines the amount of magnesium hydroxide that is formed. A recent study of magnesium flocculation of the marine microalgae *Dunaliella* also proposed to control flocculation efficiency and sludge volume by dosage of base rather than by targeting a specific pH value [28].

4. Conclusions

From our experiments, it is clear that the optimum pH for magnesium flocculation is dependent on the magnesium concentration in the medium and the microalgal biomass concentration. In media with a magnesium concentration higher than 5 mM, a small increase in pH above the optimum results in massive precipitation of magnesium hydroxide and a large increase in the sludge volume. It is thus a challenge to optimize magnesium flocculation by targeting a specific pH value. In waters with a high magnesium concentration, magnesium acts as a buffer that absorbs hydroxides. As a result, the sludge volume increases only if a large amount of base is added, much more than the amount to achieve the optimum pH for flocculation. Therefore, rather than targeting a specific pH level, the sludge volume can more easily be controlled by controlling the dosage of base.

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Tables and Figures captions

Table 1. Flocculation efficiency, sludge volume and residual magnesium concentration in the medium as a function of pH and initial magnesium concentration in the medium. Replicate experiments were only carried out for pH 11 and 10 mM magnesium (n = 3).

Fig. 1. The flocculation efficiency of *Chlorella vulgaris* at different pH levels in media containing different magnesium concentrations.

Fig. 2. Zeta potential of *Chlorella vulgaris* cells as a function of pH in medium without magnesium and with magnesium (0.75 mM). Flocculation occurred between pH 11 and 11.5 in the medium with magnesium while no flocculation occurred in the medium without magnesium.

Fig. 3. The flocculation efficiency of *Chlorella vulgaris* as a function of biomass concentration, at pH 10.5, 11 and 12 in medium with low (0.5 mM) and higher (1.5 mM) magnesium concentration.

Fig. 4. Relation between the sludge volume and the amount of precipitated magnesium in the flocculation experiments shown in Table 1. Magnesium concentrations varied between 5, 10 and 15 mM while pH varied between 10.5, 11 and 12.

Fig. 5. Variation in flocculation efficiency, sludge volume and dose of sodium hydroxide added as a function of pH in medium with low (0.15 mM) and high (7.5 mM) magnesium concentration.

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Table 1. Flocculation efficiency, sludge volume and residual magnesium concentration in the medium as a function of pH and initial magnesium concentration in the medium. Replicate experiments were only carried out for pH 11 and 10 mM magnesium (n = 3).

Magnesium (mM)	pH	Flocculation efficiency (%)	Sludge volume (mL)	Residual magnesium (%)
5	10.5	89.9	20	81
5	11	95.6	127	11
5	12	96.8	192	2
10	10.5	88.9	14	53
10	11	97.4 (±1.1)	283 (±8)	14 (±0.8)
10	12	96.8	286	1
15	10.5	98.2	118	82
15	11	99.4	488	3
15	12	98.7	440	1

Figure 1

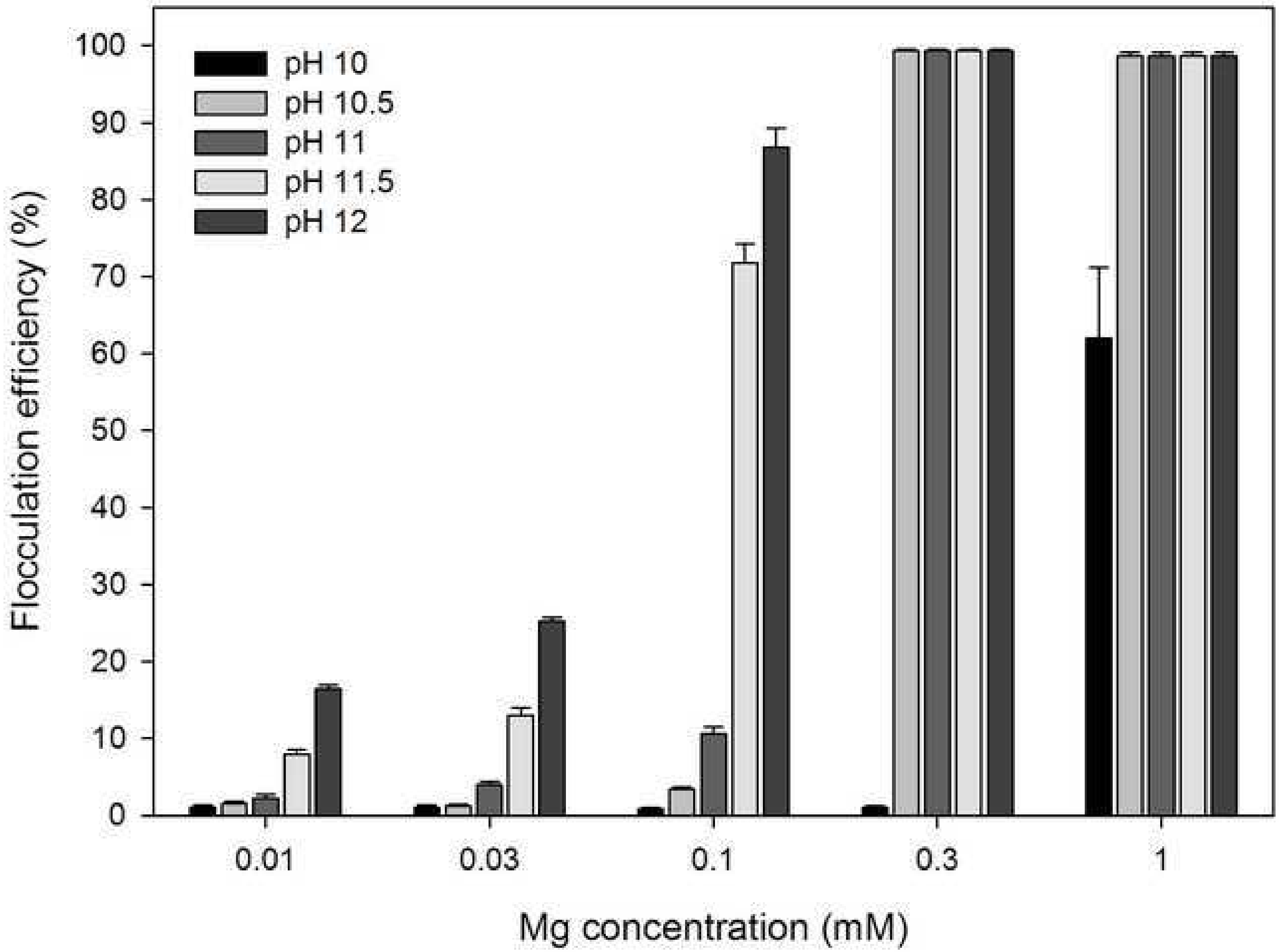

pH 10
pH 10.5
pH 11
pH 11.5
pH 12
Flocculation efficiency (%)
0
10
20
30
40
50
60
70
80
90
100
0.01
0.03
0.1
0.3
1
Mg concentration (mM)

Figure 2

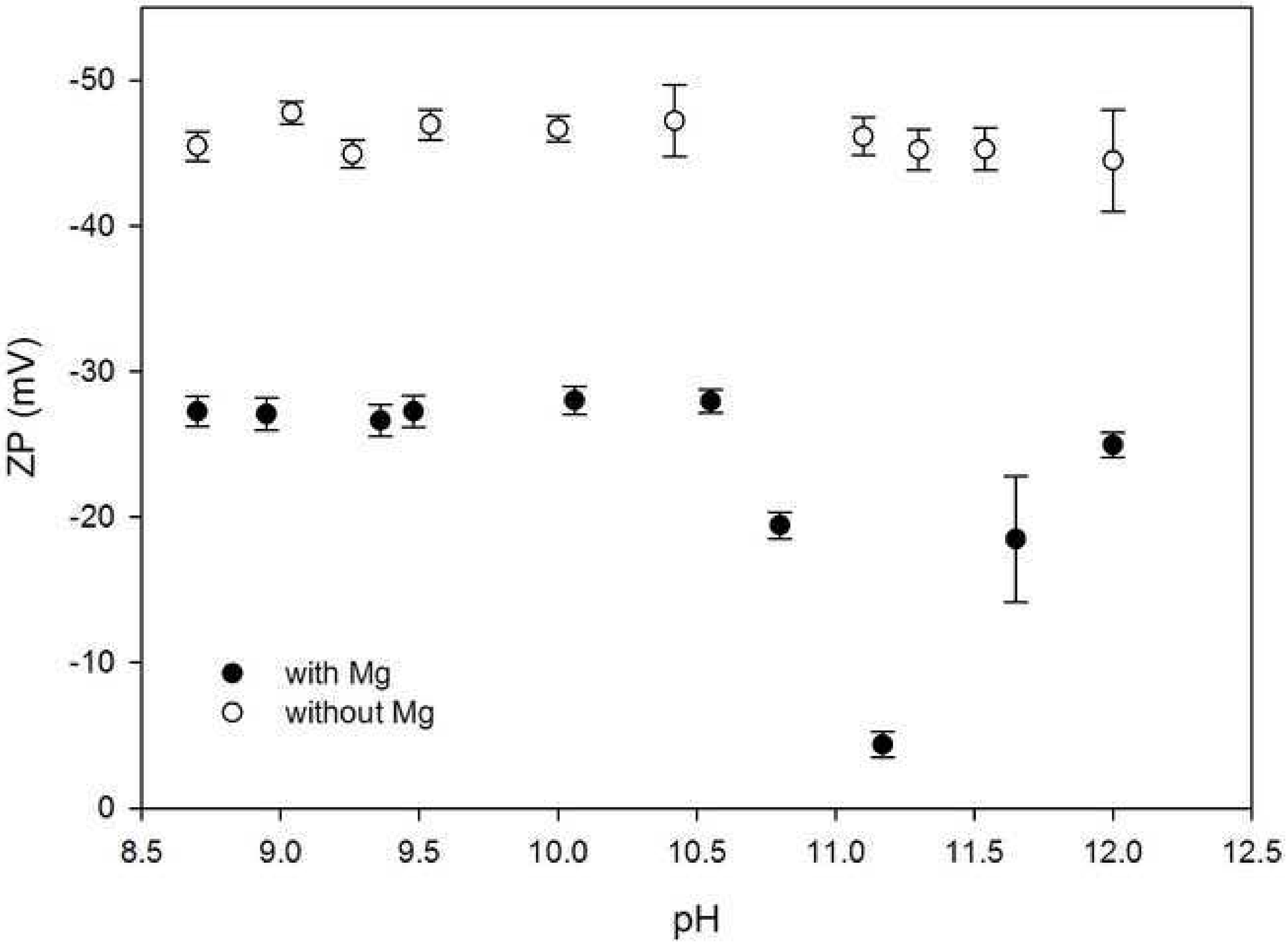

ZP (mV)
-50
-40
-30
-20
-10
0
pH
8.5
9.0
9.5
10.0
10.5
11.0
11.5
12.0
12.5
● with Mg
○ without Mg

Figure 3

Click here to download high resolution image

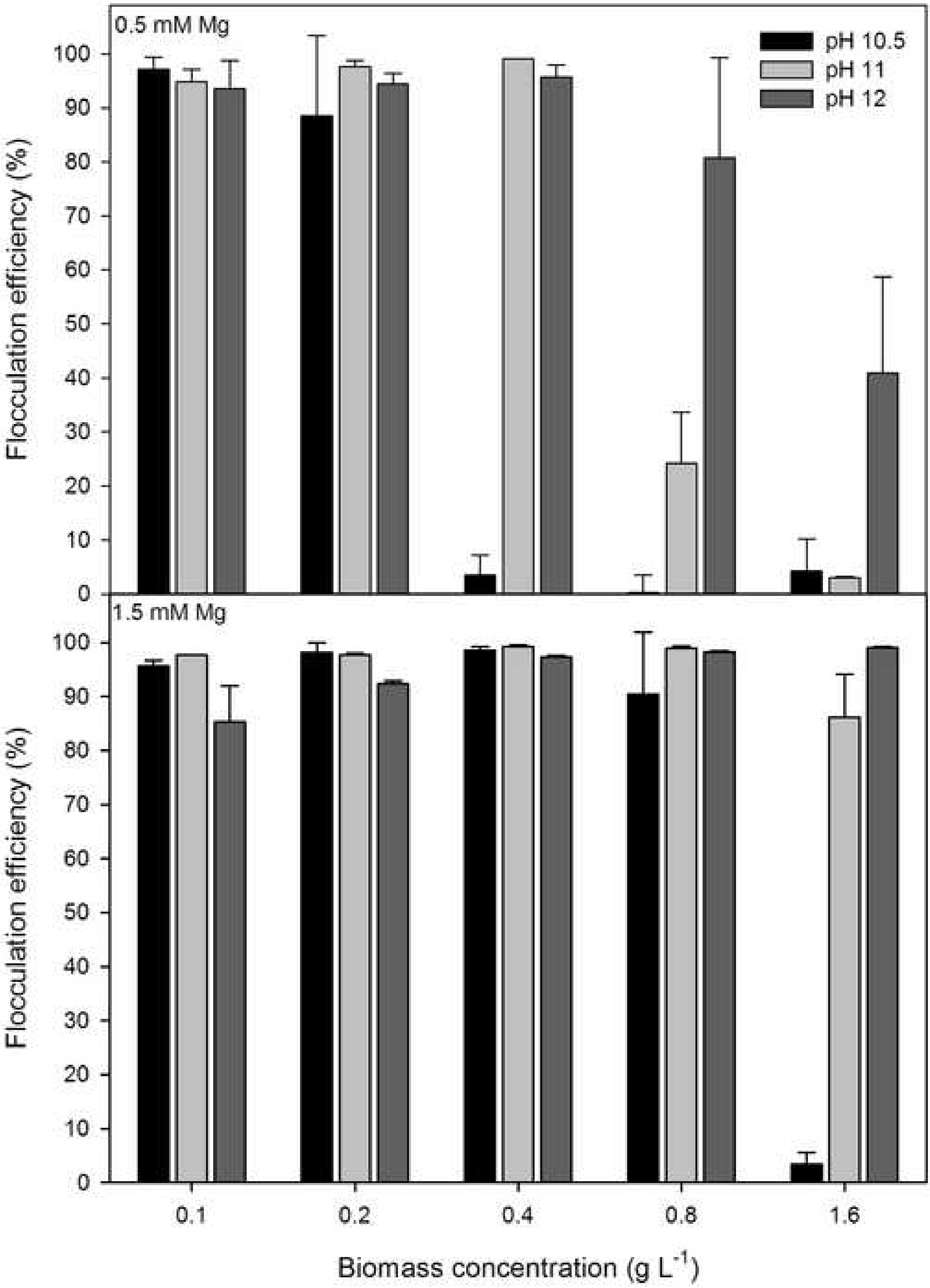


Figure 4

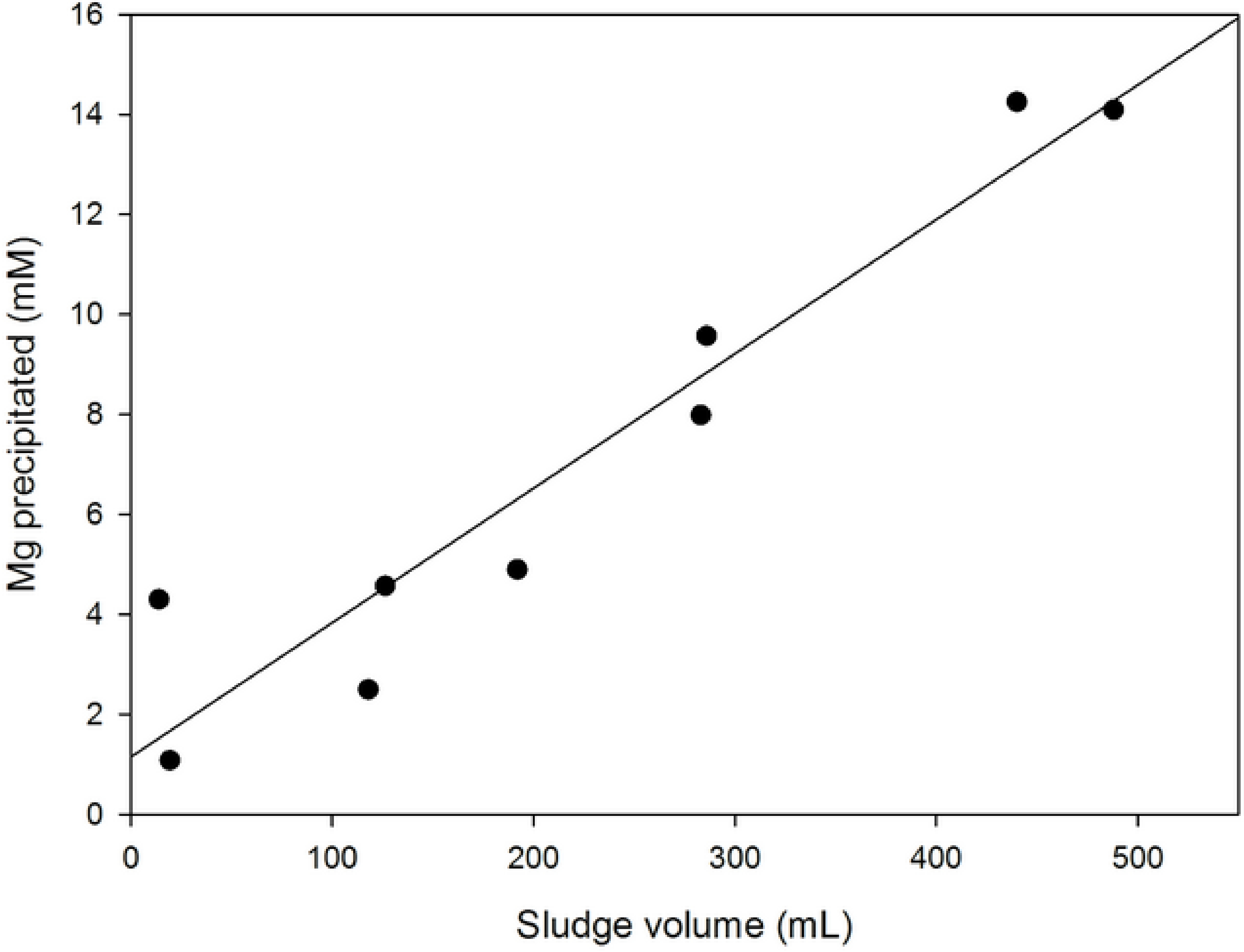
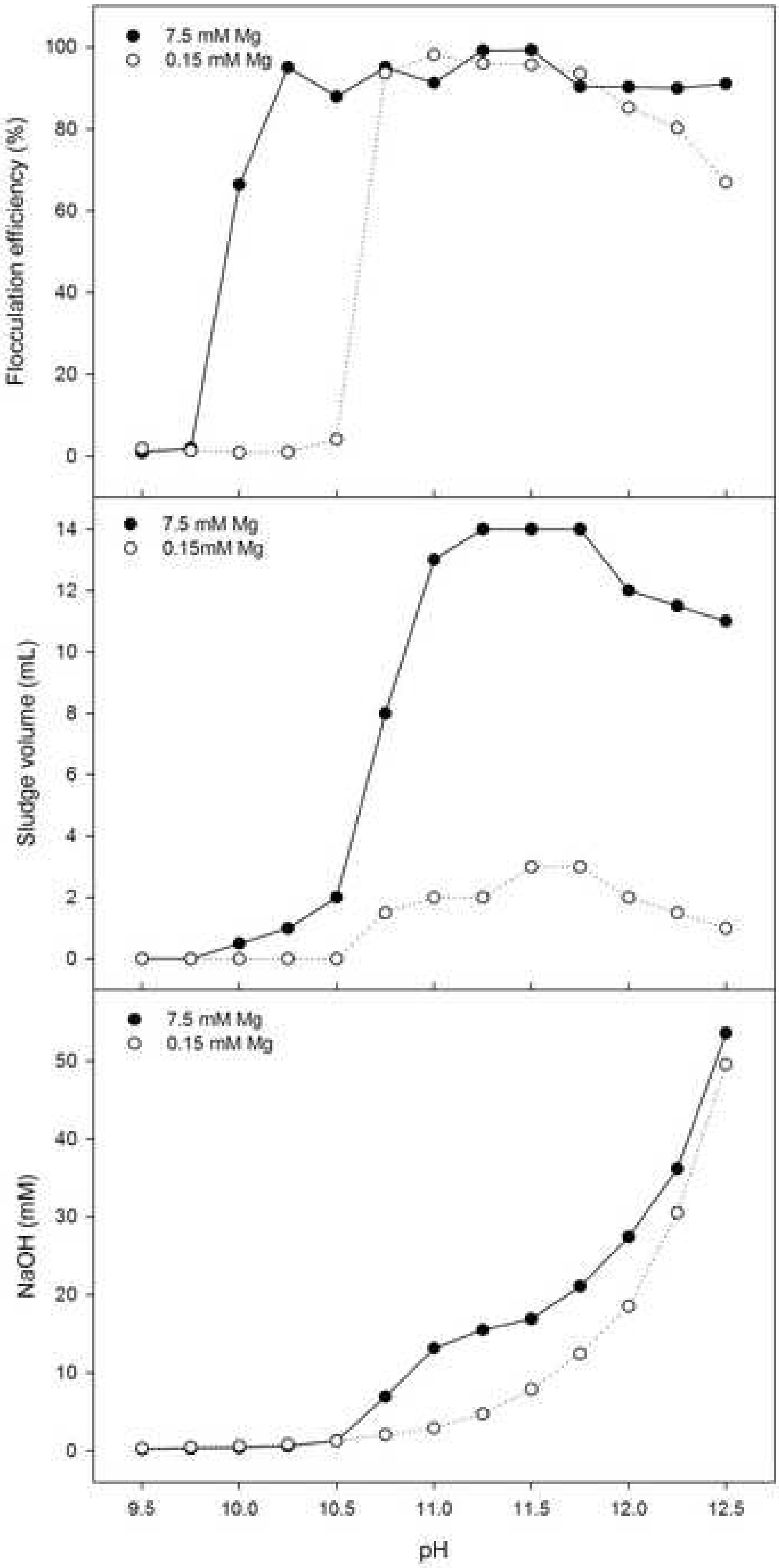

Mg precipitated (mM)
0
2
4
6
8
10
12
14
16
Sludge volume (mL)
0
100
200
300
400
500

Figure 5


7.5 mM Mg
0.15 mM Mg
Flocculation efficiency (%)
0
20
40
60
80
100
7.5 mM Mg
0.15mM Mg
Sludge volume (mL)
0
2
4
6
8
10
12
14
7.5 mM Mg
0.15 mM Mg
NaOH (mM)
0
10
20
30
40
50
9.5
10.0
10.5
11.0
11.5
12.0
12.5
pH