MICROALGAE RECOVERY VIA ALKALINE FLOCCULATION IN A STABILIZATION POND

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ABSTRACT

Purpose: This research study aimed to investigate alkaline flocculation for the recovery of biomass found in wastewater, with the presence of microalgae collected from the maturation pond of a Wastewater Treatment Station in Brazil.

Methods: Alkaline flocculation assays were conducted up to 24 hours after samples were collected due to the nature of alkaline flocculation, which may occur spontaneously if samples are stored for too long. Flocculation was obtained using the three proposed bases: sodium hydroxide, calcium hydroxide and sodium carbonate.

Results and conclusion: The efficiency tended to increase with increased pH. Sodium hydroxide as the base produced better results for pH values 11 and 12.

Research implications: Possibility of dual use of microalgae, in bioremediation and, in doing so, in the production of biomass that can later be converted into biofuels.

Originality/value: The search for sustainable technologies and the possibilities of use presented by microalgae is clear about the need to develop cultivation and harvesting methods that are economically viable.

Keywords: Autoflocculation, Biomass, Wastewater Reuse, Environmental Sustainability.

RECUPERAÇÃO DE MICROALGAS POR MEIO DE FLOCULAÇÃO ALCALINA EM LAGOA DE ESTABILIZAÇÃO

RESUMO

Finalidade: Esta pesquisa teve como objetivo investigar a floculação alcalina para recuperação de biomassa encontrada em águas residuárias, com a presença de microalgas coletadas na lagoa de maturação de uma Estação de Tratamento de Esgoto no Brasil.

Métodos: Os ensaios de floculação alcalina foram realizados até 24 horas após a coleta das amostras devido à natureza da floculação alcalina, que pode ocorrer espontaneamente se as amostras forem armazenadas por muito tempo. A floculação foi obtida utilizando três bases propostas: hidróxido de sódio, hidróxido de cálcio e carbonato de sódio.

Implicações da pesquisa: Possibilidade do uso duplo das microalgas, na biorremediação e, ao fazê-lo, na produção de biomassa que pode ser posteriormente convertida em biocombustíveis.

Originalidade/valor: A busca de tecnologias sustentáveis e das possibilidades de uso apresentado pelas microalgas é notório a necessidade do desenvolvimento de métodos de cultivo e colheita que sejam economicamente viáveis.

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1 INTRODUCTION

Microalgae are studied for their versatility, as they can be used in many applications. In the recent decades, various applications of microalgae and their subproducts have been developed and improved, including for sustainable wastewater treatment, aquiculture, and animal feed or even in the production of fertilizers, biofuels for the green circular economy (Alazaiza et al., 2023; Alazaiza et al., 2022), and several chemical compounds, contributing to environmental sustainability (Roy et al., 2022). In this context, innovation emerges as a new approach to environmental sustainability, based on the alignment between economic growth and environmental resource management (Salgado and Franchi, 2023). Microalgae have been explored in the field of flocculation as alternatives to petroleum-based compounds for better sustainability. Culture systems and biomass recovery have also been developed, to reduce costs and boost product efficiency (Roy et al., 2022; Bhatt et al., 2022; Ma et al., 2023; Moreira et al., 2022).

Two of the biggest challenges of microalgae are culture and biomass recovery. The methods currently employed show good efficiency in the laboratory or pilot scale, but still need to be improved so that they are economically viable and reliable for large-scale implementation. In general, cultivation demands the addition of nutrients, the use of water and a high energy expenditure. The latter requires investments in raw materials and energy and can become contaminate by unwelcome chemical compounds (Alazaiza et al., 2023; Milano et al., 2016; Show et al., 2013).

Given the current scenario of search for sustainable technologies and the potential use presented by microalgae, the need to develop cultivation and harvesting methods that are economically viable is notorious. In this sense, taking advantage of the microalgae used in the sewage treatment process has a lot of potential, acting on two fronts: in the production of biomass and the treatment of effluent. This co-cultivation eliminates the need to add nutrients, CO₂ and other compounds, taking advantage of those normally present in sewage, in addition to using wastewater to the detriment of the use of natural water sources (Christenson and Sims, 2011).

Even though no universal method meets all the requirements of biomass recovery (which may vary according to culture medium, species involved, ability of spontaneous flocculation, etc.), the combination of flocculation and sedimentation probably requires the least energy consumption (Milledge and Heaven, 2013; Matter et al., 2019). Flocculation may be obtained by different mechanisms: coagulation-flocculation, physical flocculation, bioflocculation, and self-flocculation. Microalgal have been explored in the field of flocculation due to the need for new products obtained from sustainable alternatives to petroleum-based compounds. The main future challenges in the bioremediation sector are the production of exopolysaccharides on an industrial scale to provide environmental added value (Ma et al., 2023; Matter et al., 2019; Vandamme et al., 2013).

Alkaline flocculation or self-flocculation is a natural phenomenon observed in microalgae culture whenever the pH naturally increases due to a reduction in available CO₂ (Matter et al., 2019), influenced by the presence of calcium, magnesium, and phosphates in a given medium. The reaction between these compounds in a medium with high pH leads to the
formation of flakes that use microalgae cells as a support medium. This phenomenon may be artificially induced, either by interrupting the CO₂ supply (possible in photobioreactors) or by adding basic compounds, such as sodium hydroxide, calcium hydroxide or potassium hydroxide (Moreira et al., 2022; Vandamme et al., 2012; Knuckey et al., 2006).

Therefore, if the recovery of biomass present in sewage treatment is interesting because it eliminates many of the expenses involved in the cultivation, whether with nutrients or energy, on the other hand, environmental samples like this one present specificities such as the presence of several species of microalgae and of other microorganisms, which may impact the final result. Therefore, this research study aimed to investigate alkaline flocculation for the recovery of biomass found in a wastewater volume, with the presence of microalgae collected from the maturation pond of a Wastewater Treatment Station (WTS) in Brazil.

2 TEORETICAL FRAMEWORK

The use of biomass from microalgae is one of the alternatives with the greatest potential to replace the current energy model, based on fossil fuels. The potential use of biofuels from microalgae brings advantages in carbon sequestration, lower environmental impact and reduced competition with food production, however, more studies are still needed to make this technology economically viable to compete with the use of fuels fossils (Demirbas, 2011; Schenk et al., 2008). Also, organisms that inhabit aquatic environments, such as algae, among others, can be targets of environmental monitoring (Barros et al., 2023).

In general, two forms of solid-liquid separation are used to recover biomass. A first phase of collecting the suspended material, concentrating it, and another of removing the aggregated biomass. In this sense, flocculation can be used as a preliminary method, aggregating the particles, for subsequent use of centrifugation, sedimentation, flotation or filtration for final separation of the biomass from the liquid médium (Brennan and Owende, 2010; Grima et al., 2003).

Several flocculation processes are studied to achieve an economically efficient recovery of biomass (coagulation-flocculation, physical flocculation, natural flocculators, bioflocculation, autoflocculation, etc.). Flocculation technologies present themselves as a simple mechanism with the potential to harvest algal biomass in an economically viable way and at the lowest possible energy cost (Gerardo et al., 2015; Vandamme et at., 2013).

Techniques that start from a more natural, biological basis are the most promising due to efficiency, cost and minimal environmental impact caused. There are four main forms of flocculation that are used for this purpose: alkaline flocculation, bioflocculation, coagulation-flocculation and physical flocculation (Wan et al., 2015).

Alkaline flocculation and bioflocculation are natural techniques, which can occur naturally, while coagulation-flocculation and physical flocculation use artificial mechanisms to form flocs. The ideal characteristics of the flocculation process include the ability to recover biomass without leaving residue, enable subsequent sedimentation, allow the reuse of wastewater in new crops and cause the least possible environmental impact (Chen et al., 2012).

The morphology and properties of microalgae cells vary depending on the growth phase and the induction of alkaline flocculation. Understanding these changes is important to determine the best time for biomass recovery, improving the efficiency of the method (Salim et al., 2013). Once the process occurs without the addition of chemical compounds, the wastewater, at the appropriate pH, can be recirculated to induce alkaline flocculation again (Sirin et at., 2012).
3 METHODS

The microalgae were collected from the WTS located in Goianira, Goiás (Brazil). This WTS has a system composed of anaerobic, facultative, and maturation ponds operating serially, as shown in Figure 1. The anaerobic pond is 5 meters deep and 1380 m² of surface area; the facultative is 2 meters deep and 8569 m² of surface area, while the maturation pond is 1.5 meters deep and 6499 m² of surface area. The sewage processing station was designed to handle a flow of 26.62 Ls⁻¹. Samples were collected at the maturation pond, with characteristics listed in Table 1.

The effluent was collected close to the spillway at the exit channel of the maturation pond. Samples were conditioned in 20 L plastic containers. Four collections were carried out throughout the dry season. Samples were collected in the morning (from 8 to 10 am) during August and September.

Alkaline flocculation assays were conducted up to 24 hours after samples were collected, because alkaline flocculation may occur spontaneously if samples are stored for too long. Thus, possible medium changes or even biomass fraction sedimentation were duly prevented.

![Figure 1. Wastewater Treatment Station in Goianira](image)
Source: Bing, 2023.

### Table 1. Pond characterization.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (mgCaCO₃.L⁻¹)</td>
<td>97</td>
<td>14.46</td>
</tr>
<tr>
<td>Total phosphorus (mg.L⁻¹)</td>
<td>0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>Orthophosphate (mg.L⁻¹)</td>
<td>0.23</td>
<td>0.06</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>0.167</td>
</tr>
<tr>
<td>Turbidity (TNU)</td>
<td>71.83</td>
<td>6.49</td>
</tr>
<tr>
<td>N-NH₃ (mg.L⁻¹)</td>
<td>49.09</td>
<td>18.79</td>
</tr>
<tr>
<td>COD (mg.L⁻¹)</td>
<td>167</td>
<td>12.72</td>
</tr>
</tbody>
</table>

pH: hydrogen potential. TNU: nephelometric turbidity units. COD: chemical oxygen demand.
Source: Prepared by the authors (2023).

Samples were separated into six 1 L volumes and submitted to jar testing, in which alkaline flocculation was induced by a rise in pH, adjusted with the addition of solutions containing sodium hydroxide (NaOH), calcium hydroxide (Ca(OH)₂) or sodium carbonate.
(Na$_2$CO$_3$). Five pH values were tested: 8, 9, 10, 11, and 12. The sixth sample was kept unchanged as the control. Therefore, a total of 18 samples were tested for each collection done totaling 72 samplings, considering four collections carried out. Solutions with 0.5 M and 1.0 M molar concentration were selected for NaOH and Na$_2$CO$_3$ samples, and those with 0.5 M were used for Ca(OH)$_2$ samples.

Following pH adjustment for the predetermined values, samples were jar tested. They were stirred for 1 min at 80 rpm, followed by 15 min at 30 rpm, and allowed to stand for 15 min for sedimentation to occur (Matter et al., 2019; Mayers et al., 2018; Oliveira et al., 2022; Sukenik and Shelef, 1984). Once the assay was completed, samples were collected in cubes, and absorbances were read via a spectrophotometer.

The pH values were obtained by electrometric analysis. The efficiency of the method was estimated by calculating and comparing absorbance in spectrophotometer at 420 nm wavelength between adjusted samples and control (Roy et al., 2022). Samples for determining absorbance were collected from the medium of each clear zones in each jar, which were measured individually. Efficiency calculation was performed by Equation 1 (Vandamme et al., 2012).

$$E_a = \frac{OD_i - OD_f}{OD_i}$$ (1)

In with:

$E_a$ is the alkaline flocculation efficiency;

$OD_i$ represents the absorbance of the control volume after 30 minutes of sedimentation (dimensionless); and

$OD_f$ is the absorbance of the volume of interest, with adjusted pH, after the assay (dimensionless).

Comparison between efficiencies elucidated the ideal pH value for high pH-induced flocculation to occur, hence indicating the best base for pH induction.

The data obtained were subjected to analysis of variance (ANOVA) (P≤0.05), considering the means efficiency of the three bases used and the different pH values as sources of variation (Excel 10.0).

4 RESULTS AND DISCUSSION

The results obtained are presented below considering the variation in the concentration of microalgae, alkaline flocculation test and sedimentation.

4.1 Variation in the Concentration of Microalgae

During the data collection period, the volume reduced in the maturation pond at the WTS in Goianira due to the removal of a spillway exit channel. The color also changed from green to greenish-yellow, probably indicating an increase in crustaceans, rotifers, and protozoa, all of which feed on microalgae (Roy et al., 2022; Iram et al., 2012; Ding et al., 2021).

These changes in the pond impacted the mean absorbance determined on the 420 nm wavelength, indicating reduced microalgae concentration. Absorbance was used as an indirect measure of microalgae concentration. Mean absorbance values went from 0.597 to 0.243 during the period in question but increased again later (Table 2).
Table 2. Variation in the concentration of microalgae in the pond over the collection period.

<table>
<thead>
<tr>
<th>Collection date</th>
<th>Absorbance (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/08/2019</td>
<td>0.597</td>
</tr>
<tr>
<td>06/09/2019</td>
<td>0.243</td>
</tr>
<tr>
<td>10/09/2019</td>
<td>0.401</td>
</tr>
<tr>
<td>12/09/2019</td>
<td>0.379</td>
</tr>
</tbody>
</table>

Source: Prepared by the authors (2023).

The change in the volume of the pond did not lead to changes in pH, phosphorus, or hardness values that were sufficient to inhibit alkaline flocculation. However, biomass removal efficiency varied due to medium changes. Therefore, as a result of changes in the behavior of the pond, the effects of alkaline flocculation were verified in pH values and the influence of microalgae concentration on the efficiency of each base.

4.2 Alkaline Flocculation

Flocculation was obtained using the three proposed bases. The efficiency tended to increase with increased pH (Figure 2). This tendency remained independent from the sample evaluated. However, NaOH and Ca(OH)$_2$ achieved significantly higher efficiency values than those obtained with Na$_2$CO$_3$.

In addition to low efficiency, another limitation compared to the other bases was that the ability of Na$_2$CO$_3$ to induce pH is compromised by the solution volume required to increase pH for values greater than 10.

The pH values of 8, 9, and 10 yielded low or zero efficiency in the majority of assays, with the exception of Ca(OH)$_2$ samples. This is similar to results found by Vandame et al. (2013), who did not observe any flake formation up to pH 10. In pH 11 and 12, their efficiency reached values higher than 50% and lower than 82% (using Ca(OH)$_2$ and NaOH as pH inducers).

According to the analysis proposed by Vandame et al. (2013), the use of NaOH and Ca(OH)$_2$ in pH induction requires the least amount of base for alkaline flocculation to occur in terms of milligrams per gram of biomass (9 mg·g$^{-1}$ and 12 mg·g$^{-1}$, respectively). Therefore,
Ca(OH)$_2$ was the best compound studied because, in addition to inducing flocculation at pH values, it also has advantages related to cost and lower risk of accidents.

Comparing the general behavior, Spilling et al. (2010) obtained efficiency results of about 70% to 80% by increasing pH naturally (in salt water, via the increment in magnesium concentration) in the recovery of microalgae kept in a controlled culture after 1 h of sedimentation. Studies that employed bases achieved, efficiency values higher than 90%, applying different parameters, such as greater sedimentation time, pure microalgae cultures, high magnesium concentration, salt water, and controlled medium (Vandamme et al., 2012; Oliveira et al., 2022; Besson and Guiraud, 2013; Salim et al., 2013).

Besson et al. (2013) obtained values over 90% in the recovery of microalgae cultured in ponds. Sodium hydroxide was used in a pond designed for algae culture and Na$_2$CO$_3$ in a facultative pond for wastewater treatment, respectively. In both cases, calcium and magnesium concentrations were increased to an ideal level before flocculation. The presence of calcium and magnesium is crucial for alkaline flocculation to take place, and greater availability of these ions directly impacts efficiency (Matter et al., 2019; Vandamme et al., 2012; Şirin et al., 2012; Schlesinger et al., 2012).

Calcium hydroxide yielded the best results, in line with results obtained by Spilling et al. (2010). This was the only base that showed mean efficiency higher than 40% in pH 10 and higher than 70% in pH 12. On the other hand, its use also led to higher sludge production. Lower efficiency in this research study was expected due to the very nature of maturation ponds. Concentrations of orthophosphates, calcium and magnesium are lower due to the prior treatment.

Phasey et al. (2017) highlighted that the calcium and phosphate concentrations required to induce alkaline flocculation in medium samples are much higher than those observed in the literature for controlled medium samples. On the other hand, the community of different microorganisms is higher in medium samples and microorganisms with negative surface loads end up “competing” with microalgae in flake formation during alkaline flocculation.

In addition to these factors, alkaline flocculation efficiency varies among species as well as pH values, culture systems, growth phase and medium compounds, among others. These differences may lead to variation in microalgae recovery and make it difficult to compare results (Bracharz et al., 2018; Maji, G., 2018).

### 4.3 pH Induction by Sodium Hydroxide (NaOH)

The use of NaOH as the base yielded better results for pH values 11 and 12. Considering the mean, pH 8 and 9 were not efficient or led to low-efficiency values, up to 4%. A pH 10, obtained maximum efficiency at 14.13%.

The maximum efficiency registered with NaOH was 55.16% (49.63% on average) for pH 12 (Figure 3), where the dots represent the outliers values and the crosses represent the mean value. The rise in pH was directly related to increased efficiency. The literature on the topic showed similar results, with low flake formation in pH values less than 10, zero flake formation at pH 8, and 30% to 50% efficiency at pH 12.

Vandamme et al. (2012) obtained efficiencies greater than 95% by using NaOH to increase pH in samples to 11 and 12. This efficiency was obtained in a controlled sample with only one species of interest. Though these authors obtained a higher maximum efficiency value, the alkaline flocculation behavior they verified was similar to this research study, with low or zero efficiencies for pH 8, 9 and 10 as well as higher values for pH 11 and 12.

Besson et al. (2013) were able to obtain 90% efficiency using NaOH for these pH values, as well as in a controlled sample and salt water (by increasing magnesium concentration in the medium). Similarly, Oliveira et al. (2022) and Sirin et al. (2012) employed controlled samples...
and known species and obtained values of over 89% as from pH 9 (including a 98% maximum efficiency for pH 11) using NaOH. The authors focused their analysis on sedimentation, so that these results were reached within at least 1 h in which samples were allowed to stand, following pH induction and flake formation.

Figure 3. Efficiency (%) of biomass recovery in all samples with sodium hydroxide.
Source: Prepared by the authors (2023).

Efficiencies of over 98% from pH 8 and up were observed by Sukenik and Shelef (1984) using NaOH as inductor. In this research study, microalgae of interest were also cultured in ponds, though the latter were designed for microalgae production rather than wastewater treatment. The authors highlighted the importance of controlling chemical compounds found in growth media to maximize the effect of alkaline flocculation.

Mayers et al. (2018) verified over 95% efficiency for the recovery of biomass cultured in salt water using NaOH as an inductor for lower pH values (from 8.6 to 9.7). However, this was obtained by combining the addition of a base with nutritional stress. In this case, alkaline flocculation occurred alongside spontaneous flocculation of certain species, hence increasing total efficiency. The authors contend that pH (and, consequently, alkaline flocculation) was not the central element responsible for high efficiency, given that there was no relevant precipitation of calcium and magnesium compounds, and assigned spontaneous flocculation a more relevant role in their findings.

Table 3 and Figure 3 present the means of all efficiency-related data. However, if results are separated by grouping data pertaining to the period in which the conditions in the pond changed, a considerable difference is noted among mean efficiency values (Table 3). Data showed considerable variation in mean efficiency values during removal. General behavior remained, with the rise in efficiency conditioned to a rise in pH, particularly for pH 11 and 12. Nevertheless, efficiency in algae biomass removal was more than two times higher in the most concentrated samples.

As previously mentioned, alkaline flocculation is closely linked to the presence of orthophosphates, calcium and magnesium found in the water (Matter et al., 2019; Vandamme et al., 2012). The mean concentration of orthophosphates was higher within the period of lowest microalgae concentration (mean values of 0.29 mg.L⁻¹ and 0.18 mg.L⁻¹, respectively). Hardness was also higher during this period (103.0 mg CaCO₃.L⁻¹ and 85.33 mg CaCO₃.L⁻¹, respectively).

Given orthophosphate, calcium, and magnesium concentrations, higher efficiency was expected during the period in question, but initial concentration of microalgae was ultimately a
determining parameter in alkaline flocculation efficiency. Notwithstanding, variation in microalgae concentration alone does not explain this abrupt change in the efficiency.

### Table 3. Mean efficiency by pH range and absorbance (NaOH).

<table>
<thead>
<tr>
<th>pH range</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean efficiency (%)</td>
<td>0.0</td>
<td>0.65</td>
<td>17.80</td>
<td>40.29</td>
<td></td>
</tr>
<tr>
<td>Initial mean absorbance 0.400 (420nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean efficiency (%)</td>
<td>0.86</td>
<td>5.41</td>
<td>25.76</td>
<td>49.63</td>
<td></td>
</tr>
<tr>
<td>Initial mean absorbance 0.244 (420nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean efficiency (%)</td>
<td>0.0</td>
<td>0.0</td>
<td>5.86</td>
<td>16.94</td>
<td></td>
</tr>
<tr>
<td>Source: Prepared by the authors (2023).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The color of the pond during the period of lowest microalgae concentration is another factor that helps shed light on variation in efficiency. The yellowish color of the water indicates the presence of other microorganisms such as bacteria, fungi, rotifers and protozoa (Oliveira et al., 2022; Iram et al., 2012). Higher concentration of microorganisms with negative surface loads, combined with a lower microalgae concentration, may reduce the efficiency of microalgae removal (despite alkaline flocculation occurring normally), since compounds based on precipitate calcium and magnesium are also linked to these microorganisms (Mayers et al., 2018; Besson et al., 2013).

Among bases analyzed by Vandame et al. (2012) and Mayers et al. (2018), NaOH is the one that requires the lowest amount of mols for pH induction. Maji et al. (2018) contend that, despite a lower mass demand, NaOH may reduce lipid potential in some freshwater microalgae species. During the experiment, sedimentation of flakes formed via NaOH produced a smaller amount of sludge than the one formed via Ca(OH)₂ (Figure 4). Na₂CO₃ did not obtain efficiency values for a viable comparison with the other bases. Moreover, due to a lower precipitate amount, flakes are easier to observe through when NaOH is employed.

![Figure 4](image-url)  
**Figure 4.** Flakes formed after pH induction with NaOH.  
**Source:** Prepared by the authors (2023).
The amount of sludge generated is an important advantage, since a later use of the biomass may require the separation of the compounds present. Thus, the use of sodium hydroxide seems to cause less contamination of the biomass obtained, although at the cost of lower efficiency than the use of calcium hydroxide.

4.4 pH Induction by Sodium Carbonate (Na₂CO₃)

Among the three bases, Na₂CO₃ was the least efficient (Figure 5). As previously stated, the solution volume required to increase pH for values over 11 compromised the entire process, hence it was impossible to reach pH 12 using Na₂CO₃ as a base. A 0.5M solution was used early on; however, once the limitation was verified, it was replaced by a 1.0M solution. Even so, induction was not possible due to the required volume, and this change in molarity did not lead to efficiency variation.

![Figure 5](image-url)  
**Figure 5.** Efficiency (%) of biomass recovery in all samples with sodium carbonate.  
**Source:** Prepared by the authors (2023).

According to Spilling et al. (2010), even though Na₂CO₃ was the cheapest base, it was unable to induce alkaline flocculation during assays (despite having reached a pH higher than 11). In this study, the highest efficiency obtained was 29.08% for pH 11, with a mean efficiency of 17.99% (Figure 5 and Table 4).

Similar to the use of NaOH, efficiency was lower during the period of lowest microalgae concentration with the use of Na₂CO₃, though the effect was less pronounced (Table 4). During this period, alkaline flocculation was only reached at pH 11, with no floculations verified in the other pH values.

<table>
<thead>
<tr>
<th>pH range</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean efficiency (%)</td>
<td>0</td>
<td>0</td>
<td>4.33</td>
<td>17.99</td>
</tr>
</tbody>
</table>

**Initial mean absorbance 0.400 (420nm)**

| Mean efficiency (%) | 0  | 1.7 | 9.67 | 21.50 |

**Initial mean absorbance 0.244 (420nm)**

| Mean efficiency (%) | 0  | 0  | 0  | 12.74 |

**Source:** Prepared by the authors (2023).
Considering the mean found at pH 11 during the period of highest microalgae concentration (21.50%), efficiency with Na$_2$CO$_3$ was close to that obtained with NaOH (25.76%). However, pH 11 was the limiting factor during assays. The sample amount required to increase pH above this value would render the method inconsistent, because, adding a considerable sample volume would ultimately dilute the sample, hence interfering directly on the absorbance reading and leading to an inaccurate efficiency value.

The use of sodium carbonate is unfeasible, and indicates the need for an adequate choice of bases. Even when reaching the pH value of 11, the efficiency was still very low. As noted, the concentrations of Ca$^+$ and orthophosphates must be considered, but without forgetting to consider how each base reacts with the medium, making the pH increase more or less efficient.

### 4.5 pH induction by Calcium Hydroxide Ca(OH)$_2$

Ca(OH)$_2$ yielded the best efficiency values for microalgae recovery of the three bases, with alkaline flocculation having been reached as from pH 9. As expected, the highest values were observed in high pH values, having peaked at 81.32% for pH 12 (Figure 6). Values obtained for pH 11 were already higher than the best results observed via NaOH and Na$_2$CO$_3$.

**Figure 6.** Efficiency (%) of biomass recovery in all samples with calcium hydroxide. 
**Source:** Prepared by the authors (2023).

As demonstrated by Mayers et al. (2018) and Beuckels et al. (2013), the highest calcium concentration in the medium ultimately requires a lower pH value for flocculation to begin. Moreover, the use of Ca(OH)$_2$ makes more Ca$^+$ ions available in the medium, hence producing greater efficiency than the other bases tested (Matter et al., 2019). However, the practical effect of this efficiency must be considered, which came with the increase in the amount of sludge generated, which may be a deficiency in the use of the base.

Phasey et al. (2017) used wastewater and agricultural effluent to test alkaline flocculation. They concluded that a natural increase in pH is not viable for alkaline flocculation and that the use of Ca(OH)$_2$ reached 93% efficiency in removal microalgae of biomass. Additionally, they highlighted phosphate removal (reaching 96%) and competitive cost in relation to conventional coagulants as further benefits of Ca(OH)$_2$.

Chen et al. (2013) dosed several compounds, among them Ca(OH)$_2$, as flocculants for the removal of a microalga species cultured in a pond. The authors verified that flocculation
efficiency was not higher than 60% for pH values below 10. For pH values 11 and 12, efficiency via Ca(OH)$_2$ reached 90% after 120 min of sedimentation. Scherer et al. (2016) obtained maximum efficiency of 20% in microalgae removal using Ca(OH)$_2$ as a flocculator, but with the dosage and not the pH value as the main parameter.

Considering the period of lowest microalgae concentration in this study, Ca(OH)$_2$ was the least affected base. The value obtained for pH 9, 10, and 11 was superior to that of the period with highest microalgae concentration (Table 5). Even in the analysis of pH 12, efficiency remained at around 70% of removal.

**Table 5.** Mean efficiency by pH range and absorbance (Ca(OH)$_2$).

<table>
<thead>
<tr>
<th>pH range</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean efficiency (%)</td>
<td>0</td>
<td>9.92</td>
<td>45.55</td>
<td>5771</td>
<td>99</td>
</tr>
<tr>
<td>Mean absorbance (0,400 nm)</td>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean efficiency (%)</td>
<td>5.03</td>
<td>9.34</td>
<td>37</td>
<td>3050</td>
<td>5972</td>
</tr>
<tr>
<td>Initial absorbance (0,244 nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean efficiency (%)</td>
<td>10.80</td>
<td>9.166</td>
<td>5170</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

**Source:** Prepared by the authors (2023).

The highest efficiency in utilizing Ca(OH)$_2$ as a pH inductor was recommended by Vandamme et al. (2012), therefore, expected. However, efficiencies observed during the period of lowest concentration were surprising, since the other bases produced a significant reduction in microalgae recovery during the same period.

As previously mentioned, orthophosphate concentration was higher during the period of changes to the overall conditions in the pond. The greater availability of orthophosphates (of the medium) and calcium (of the base) accounts for high efficiency even during the period of the lowest microalgae concentration. This finding confirms results obtained by Beuckels et al. (2013), who contend that alkaline flocculation efficiency is also linked to calcium and orthophosphate concentrations and, thus, that the higher concentration of these substances, the lower the pH required for flocculation.

Following jar testing, control samples showed little variation in absorbance values. Nevertheless, the induction of pH by Ca(OH)$_2$ led to an increase in turbidity and resulting interference in the initial absorbance reading.

### 4.6 Statistical Analysis

According to the analysis of Table 6, significant differences were verified with the different bases used in the different evaluated pH (ANOVA: double factor without repetition). The efficiency of the method was directly conditioned to the base and the pH value reached. The value of F > critical F, com $p<0.05$, indicated that there are significant differences between the different bases used in the different pH values evaluated.

**Table 6.** Analysis of variance (ANOVA) ($p<0.05$).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SQ</th>
<th>gl</th>
<th>MQ</th>
<th>F</th>
<th>$p$ value</th>
<th>critical F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>2083,112</td>
<td>2</td>
<td>1041,556</td>
<td>4,561</td>
<td>0,0476</td>
<td>4,458</td>
</tr>
<tr>
<td>Columns</td>
<td>3560,401</td>
<td>4</td>
<td>890,1</td>
<td>3,897</td>
<td>0,0481</td>
<td>3,837</td>
</tr>
<tr>
<td>Error</td>
<td>1826,952</td>
<td>8</td>
<td>228,369</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7470,466</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SQ:** quadratic sums. **gl:** degrees of freedom. **MQ:** quadratic means. **F:** F-statistic.

**Source:** Prepared by the authors (2023).
The main objective of the ANOVA performed was to determine whether the mean efficiencies of the bases used were statistically different or not, at different pH values. Thus, the null hypothesis was rejected at a certain level of significance (p≤0.05), and it was verified that there is at least one of the means of a base used that is different from the others. Determining whether the results of an ANOVA are significant involves looking at the p value associated with the calculated F statistic. The F statistic is a ratio of variances between groups and within groups. A high F value, as verified (F=4.561) indicates that the variation between the means of the three bases used is greater than would be expected by chance, which suggests that there is a significant difference between at least two of the bases used.

During the experiment, the absorbance the samples was measured before and after the start of dosing bases, even if the data was not used in Equation 1. This data indicated that the addition of the Ca(OH)$_2$ solution increased turbidity in the medium, impacting even initial absorbance value measured in the samples (Table 7).

Samples induced with Ca(OH)$_2$ ultimately showed a less apparent color than the other bases. Therefore, final absorbance may also be affected by this interference, which would underestimate the efficiency value.

Table 7. Initial and final mean absorbances (Ca(OH)$_2$).

<table>
<thead>
<tr>
<th>pH</th>
<th>control</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mean absorbance</td>
<td>0.36</td>
<td>0.410,500,620,530,47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final mean absorbance</td>
<td>0.36</td>
<td>0.360,320,220,200,11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Prepared by the authors (2023).

In accordance with Equation 1, pH values 8 and 9, are often associated with zero efficiency; however, there was actually a significant variation in absorbance in relation to the initial absorbance of samples. Although the method employed in this study was unable to confirm this, the latter could explain the similar results obtained even during the different collection periods.

Therefore, as previously mentioned, the use of calcium hydroxide showed a greater amount of sediment after alkaline flocculation (Figure 7). The sediment is also denser than that formed by adding sodium hydroxide. This behavior was expected since calcium is one of the agents involved in the process. The use of calcium hydroxide as a pH inducing base, in addition to causing an increase in pH, also makes Ca$^+$ ions available in the medium. These ions available in a supersaturated medium react with other compounds forming precipitates such as calcium carbonate (from the reaction with carbon dioxide) and calcium phosphate (from the reaction with orthophosphates), thus resulting in a greater amount of sediments (Oliveira et al., 2022; Besson and Guiraud, 2013; Schlesinger et al., 2012).

Figure 7. Sediment obtained using Ca(OH)$_2$ as pH inducing base.

Source: Prepared by the authors (2023).
On the other hand, the presence of these compounds together with the biomass can make the use of this base unfeasible, depending on the expected use of the biomass. All the compounds that sedimented can act as contamination of the biomass, depending on the final use. On the other hand, it is a positive effect when considering only the treatment of the effluent, generating a final liquid that is more clarified compared to the use of sodium hydroxide.

5 CONCLUSIONS

The efficiency of the method was directly conditioned to the base and the pH value reached. Calcium hydroxide achieves the best efficiency in recovering biomass and sediment volume among the bases observed. In addition to the bases, the pond operating conditions can vary and directly impact the results obtained from alkaline flocculation. The relationship between the concentration of microalgae and that of other microorganisms could be determinant in the increase or reduction of efficiency. Therefore, the use of alkaline flocculation for the recovery of microalgae in maturation ponds might be a viable alternative source of lipids for different uses and biomass environmental reuse. The efficiency is close to that of other studies and, with some modifications, it can present similar results at a lower cost.

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