The effects of three different flocculants on the harvest of microalgae Chlorella vulgaris

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Abstract. Today, the value of microalgae has been found and used, especially in the production of biodiesel. But there is one problem to be solved, it is the problem of microalgae harvesting. In order to study the flocculant most suitable for Chlorella, this paper compared the recovery effect of three types of flocculants on Chlorella vulgaris (C. vulgaris), in the same conditions of flocculation. In this study, the absorbance measured by UV spectrophotometer was used to indicate the concentration of C. vulgaris culture, so as to calculate the recovery rate. Compare recovery rate with different conditions. Such as flocculation time, concentration of flocculant, concentration and pH of C. vulgaris culture medium. The experimental results show that the recovery rate of polychloral chloride as a flocculant is the best, and it can reach more than 95%, only considering flocculation efficiency. Taking into account the environmental safety factors, calcium hydroxide as a flocculant for the collection of C. vulgaris is the best. But, the flocculation effect of calcium hydroxide is relatively weak, and the best flocculation effect at 0.9g/L is 86%.

1. Introduction
Today, fossil fuels are decreasing or lacking with the development of the global economy. A large number of fossil fuel combustion lead to increased emissions of harmful gases such as carbon dioxide and sulfur dioxide. The microalgae cell contains rich oil, which is an important raw material for producing biodiesel and has good application prospect. Biodiesel is a renewable energy with low ash content. After combustion, SO₂, NO and dust emissions are smaller than fossil fuels, so it is an ideal clean carbon fuel [1]. However, the harvest of microalgae is a key issue in the industrialization of biodiesel, which is estimated to account for 20% to 30% of the total industrial production cost [2]. At present, the commonly used methods of microalgae separation and collection are centrifuge, filtration, air flotation and flocculation. From the point of view of algal cell harvesting, flocculation is the most economical and feasible method to deal with a large number of microalgae culture. As early as 1980s, the flocculation methods had been applied to the separation and collection of microalgae. Gao et al [3] studied three types of flocculants on isochrysis galbana flocculation, the results showed that the flocculation rate of alum is the fastest, the mechanisms of algal cells minimum, algae yield the highest total fat. Huang et al [4] discussed the flocculation effect of seven types of flocculating agents on the three species of microalgae, such as chaetoceros moelleri, Nannochloropsis oculata and Isochrysis zhanjiangensis. The study found that the flocculation effect of alum, zinc sulfate and polyalumimum chloride on chaetoceros moelleri was better, while aluminum sulfate had better flocculation effect on
Lsochrysis zhanjiangensis. Ding et al [5] was used to flocculate C. vulgaris with polyaluminum chloride (PAC). It was found that five different concentrations of PAC could flocculate Chlorella in 8 min, and the recovery rate was over 86%. Xue et al [6] compared the harvesting effect of nine different flocculants to Chlorella and studied the recycling of culture medium. The results showed that aluminum sulfate, ferric sulfate, ferric chloride and calcium hydroxide had better harvesting effect and grew faster in the supernatant of ferric chloride and calcium hydroxide after harvest. Lama et al [7] used ferric chloride, chitosan and alkaline flocculant to flocculate 9 types of freshwater algae and marine algae, found that flocculants had selectivity for microalgae and their flocculation cost was compared. Tawan et al [8] used flocculation to harvest microalgae, proved that the flocculation effect of aluminum sulfate was better than that of ferric chloride, and the flocculation effect had a great relationship with the biomass concentration of algal liquid. Kim et al [9] used metal flocculants (Fe$_2$(SO$_4$)$_3$) and sulfuric acid (H$_2$SO$_4$) flocculation. It was found that the reduction of pH made the sediment attached to the surface of Chlorella separated. The remaining acid solution containing iron ions can be reused, and the recovery rate was three times as high as 98%. In summary, chemical flocculation is a common method of collecting microalgae, and the flocculant is also varied. Therefore, this paper chose three types of flocculant, the study of different flocculation conditions such as flocculation time, concentration of coagulant, C. vulgaris nutrient solution concentration and pH of different impact on flocculation effect, and comparison of three types of flocculant.

2. Materials and methods

2.1. Experimental materials

The experimental C. vulgaris was cultured in the BG11 medium (tables 1 and 2), and it was cultured in the conical bottle of 1 L. C. vulgaris in 25°C constant temperature incubator develop towards the end of that growth. The C. vulgaris was cultivated for about 30 d at 25°C in a thermostat incubator equipped with fluorescent lamps, until the end of growth.

| Table 1. BG11 culture medium components. |
|------------------------------------------|
| Reagent name | Unit: g/L  |
| NaNO$_3$ (AR) | 1.5000   |
| K$_2$HPO$_4$ (AR) | 0.0400   |
| MgSO$_4$·7H$_2$O (AR) | 0.0750   |
| CaCl$_2$·2H$_2$O (AR) | 0.0360   |
| Citric acid (AR) | 0.0060   |
| Ammonium citrategreen (AR) | 0.006    |
| EDTANa$_2$ (AR) | 0.0010   |
| Na$_2$CO$_3$ (AR) | 0.0200   |
| A5 | 1.0mL |

| Table 2. A5 solution components. |
|----------------------------------|
| Reagent name | Unit: g/L  |
| ZnSO$_4$·7H$_2$O (AR) | 0.222 |
| CuSO$_4$·5H$_2$O (AR) | 0.079 |
| Na$_2$MoO$_4$·2H$_2$O (AR) | 0.21 |
| H$_3$BO$_3$ (AR) | 2.86 |
| MnCl$_2$·4H$_2$O (AR) | 1.81 |
| Co(NO$_3$)$_2$·6H$_2$O (AR) | 0.05 |
2.2. Experimental methods

2.2.1. Microalgal harvesting. Determine the biomass of *Chlorella*, according to optical density (OD) measurements and the OD/dry cell weight (DCW) relationship established for *Chlorella* [10]. Firstly, microalgal medium containing 150 mL was carried out in the 250 mL beaker. Secondly, according to the experiment, a certain concentration of flocculant was added to the beaker, set aside time after 3 min stirring. Finally, the supernatant fluid from 1 cm~2 cm from the liquid surface with a liquid gun was removed, and the absorbance value of OD was measured by ultraviolet spectrophotometer, at the wavelength of 680 nm. The harvesting efficiency was calculated based on the equation (1).

\[
\text{harvesting efficiency (\%) = } \frac{(OD_0 - OD_i)}{OD_0} \times 100
\]

Where \(OD_0\) is the OD of the initial microalgal culture and \(OD_i\) is the supernatant liquid after microalgal harvesting.

2.2.2. Analysis of single factor influence. Analysis of the effect of flocculation time on the recovery of *C. vulgaris*, take 150 mL of *C. vulgaris* culture medium and add it to the beaker of 250 mL, then add a certain concentration of flocculant and stir for 3 min at 200 rpm. Get the sample every other time, as shown in figure 1. The ultraviolet spectrophotometer is used to measure the OD of the sample, and the efficiency is calculated according to equation (1), as shown in figure 1.

![Figure 1](image1.png)

**Figure 1.** The effect of the flocculation time on the recovery of the three flocculants in the harvest of *C. vulgaris* (mixing speed =200 rpm, \(p\)H in the culture medium of *C. vulgaris* =9.6).

![Figure 2](image2.png)

**Figure 2.** The effect of the flocculation concentration on the recovery of the three flocculants in the harvest of *C. vulgaris* (mixing speed=200 rpm, \(p\)H in the culture medium of *C. vulgaris* =9.6, flocculation time: Ca(OH)\(_2\)=60 min, FeCl\(_3\)=80 min, PAC=3 min).

The effect of flocculant concentration on flocculation efficiency, In this experiment, three types of flocculants (Calcium hydroxide, polyaluminum chloride and iron trichloride) were added to the culture medium of *C. vulgaris*, according to a certain concentration gradient, and 3 min was stirred by 200 rpm. Extraction of samples from the stirred *C. vulgaris* culture solution after a certain period of time. As described in figure 2. After measuring the OD of the sample, the recovery rate of *Chlorella* was calculated according to equation (1), as shown in figure 2.

In order to study the effect of the concentration of *C. vulgaris* culture solution on the recovery, the culture liquid of *C. vulgaris* was diluted in a concentration gradient. Take 150 mL diluted *C. vulgaris* medium to the beaker and add the best concentration of flocculants, respectively. And 3 min was stirred by 200 rpm. The sample is extracted as described above, and the recovery rate is calculated.
The results were shown in figure 3.

Figure 3. The effect of the concentration of C. vulgaris culture medium on the recovery of the three flocculants in the harvest of C. vulgaris (mixing speed=200 rpm, pH in the culture medium of C. vulgaris =9.6, flocculation time: Ca(OH)₂=60 min, FeCl₃=80 min, PAC=3 min, flocculant concentration: Ca(OH)₂ = 0.9 g/L, PAC = 0.3 g/L, FeCl₃=1.0 g/L).

Figure 4. The effect of the pH of C. vulgaris culture medium on the recovery of the three flocculants in the harvest of C. vulgaris (mixing speed=200 rpm, pH in the culture medium of C. vulgaris =9.6, flocculation time: Ca(OH)₂ =60 min, FeCl₃=80 min, PAC=3min, flocculant concentration: Ca(OH)₂ = 0.9 g/L, PAC = 0.3 g/L, FeCl₃=1.0 g/L).

It is considered that the PH of C. vulgaris has an effect on the recovery, adjust the culture fluid of C. vulgaris in accordance with a certain pH gradient, using 1 M hydrochloric acid solution or sodium hydroxide. The PH of C. vulgaris culture medium was adjusted to 8, 9, 9.6 (pH of C. vulgaris culture medium), 10, 11 and 12, respectively. As described above, the sample is obtained and the recovery is calculated according to equation (1). The results are shown in figure 4.

3. Results and discussion

3.1. Effect of flocculation time on flocculation efficiency

Figure 1 shows that the harvest efficiency increases with the increase of the flocculation time. The longer the flocculation time, the more thorough the C. vulgaris cells contact with the flocculant and the C. vulgaris, resulting in the more complete settlement. And the recovery rate is also high. The recovery rate tends to be stable or even decrease with the increase of flocculation time. It can be concluded from figure 1 that the optimal flocculation time of different flocculants is different, and different concentrations of the same flocculant can make the recovery rate different. Zhang et al [11] used poly aluminum silicate sulfate (PASS) and polydimethyl diallylammonium chloride (PDMAAC) as flocculant to harvest C. vulgaris, the flocculation time also varied to 90 min and 300 min respectively. From the figure, the best flocculation time of the three flocculants is 80~100 min (calcium hydroxide), 60~80 min (trichloride), and 3~5 min (polyaluminium chloride).

Three types of flocculants found that the flocculation rate of poly aluminum chloride was the fastest. After the agitation, a large number of alum flowers were produced and there were obvious delamination. This is because the ionization of aluminum salts and ferric salts produces metal cations, which neutralize the negative charge on the surface of C. vulgaris, making C. vulgaris cells accumulate and settle. In general, the higher the charge density with the flocculant, the higher the recovery rate of C. vulgaris. However, with ferric chloride and calcium hydroxide as flocculant for collecting C. vulgaris, the sedimentation rate of C. vulgaris is slower and the recovery rate of 80% is
generally more than 1 h.

3.2. Effect of flocculant concentration on flocculation efficiency

It can be seen from figure 2 that the recovery rate of *C. vulgaris* is increasing with the increase of flocculant concentration, to a certain extent. Calcium hydroxide is used as flocculant to harvest *C. vulgaris*. When the concentration of flocculant reaches 0.9 g/L, the harvest efficiency is 86% and tends to be stable. The addition of flocculant continues to lead to high concentration of calcium hydroxide, which destroys the contents of *C. vulgaris* cells and is not conducive to further oil extraction from *C. vulgaris*. So, the best concentration of calcium hydroxide flocculating *C. vulgaris* is 0.9 g/L.

Comparing with polyaluminum chloride and ferric chloride as flocculant, when the amount of flocculant reaches a certain value, increasing dosage will cause recovery rate not only increase but also decrease. The effect of PAC on *C. vulgaris* is better and the settlement speed is faster. Adding to the concentration of 0.3 g/L, the flocculation efficiency reached 98.3% and tended to stabilize, and the harvest efficiency began to decline with the addition of 0.7 g/L to the concentration. As a flocculant, ferric chloride is used as a flocculant, which is shown in figure 2 to achieve 80.6% efficiency when the concentration reaches 1.0 g/L. It is not significant to continue to increase the flocculant, and the harvest efficiency begins to decrease until the concentration increases to 1.8 g/L. The mechanism of aluminum, iron and other metal salts flocculating *C. vulgaris* is mainly the effect of electric neutralization and adsorption Bridge [12]. The metal cation produced by ionisation and the negatively charged algal cells play an electric neutralizing role, which makes algal cells aggregate. And these metal salts can also bridge with other ions in the water to form colloids, thus creating adsorption on algae cells. When the flocculant concentration reached a certain value, the negative charge on the surface of the algae cells was completely neutralized by the metal cation. To continue to increase the flocculant, the charge on the surface of the cell becomes positive charge and increases the repulsion between cells, which leads to a decline in recovery. After collecting ferric chloride with ferric chloride, Zhang *et al* [13] found that with the increase of ferric chloride dosage, the flocculation and turbidity removal rate, floc strength, recovery rate and fractal dimension of microalgae showed a trend of increasing first and then decreasing slowly. The results of the study are in agreement with the experiment.

3.3. Effect of concentration of *C. vulgaris* culture medium on flocculation efficiency

It can be seen from figure 3 that the concentration of algal liquid has a certain effect on the recovery rate of *C. vulgaris*, and the recovery rate shows a decreasing trend with the dilution of algal concentration. Zhang *et al* [13] also believed that the higher the concentration of algal liquid, the better the flocculation effect was when the same flocculant was used. In this experiment, an equivalent amount of coagulant was added. For low concentration *C. vulgaris* culture medium, the probability of collision and agglomeration between flocculants and *C. vulgaris* cells and *C. vulgaris* cells was relatively small, and the recovery rate was low. Continue to dilute, the negative charge on the surface of algae cells is not enough to neutralize the positive charge of cationic produced by ionizing flocculants, causing positive charge to accumulate and form a certain repulsive force in the solution, resulting in a significant decrease in harvesting efficiency. The flocculation efficiency tends to be stable after a certain degree of dilution.

3.4. Effect of pH on flocculation efficiency of *C. vulgaris* culture medium

Figure 4 shows that the flocculation efficiency is better when the pH value is 10. However, the effect of pH on different flocculants also varies greatly. It can be seen from figure 4 that the effect of pH on the flocculation effect of PAC and trichloride is relatively large, but the effect on calcium hydroxide is relatively small. The change of collecting efficiency when calcium hydroxide flocculated *C. vulgaris* was very small in pH from 8 to 12 and the flocculation efficiency is the lowest when the pH is not adjusted. It is indicated that the change of PH has no effect on calcium hydroxide flocculating *C. vulgaris*, but the accumulation of *C. vulgaris* is accelerated in the stirring process, and the recovery
rate is slightly increased. Polyaluminium chloride as the flocculant, with relatively strong alkaline environment its recovery efficiency is relatively high. The results of the study are similar to those obtained by Zhang [14], they used cationic flocculant harvest microalgae found that alkaline environment (10< pH<11) helps to improve the recovery of a microalgae. Because polyaluminium chloride will produce H\(^+\) continuously in the course of hydrolysis, it is necessary to have enough alkali to neutralize acid, so the recovery rate is relatively high in alkaline environment. However, as a flocculant with ferric chloride, the recovery rate is higher when the C. vulgaris is less alkaline. Fe\(^3+\) in ferric chloride exists in the form of Fe(H\(_2\)O)\(_6\)\(^3+\) under acidic conditions. With the increase of pH value, Fe(H\(_2\)O)\(_6\)\(^3+\) hydrolysates to form various polynuclear hydroxyl complexes, and finally generates Fe(OH)\(_3\) to precipitate [15]. The various polynuclear hydroxyl complexes produced by hydrolysis have more positive charges, which can neutralize the negative charge on the surface of algal cells. Therefore, the recovery rate is relatively high. When the solution is too alkaline, Fe(OH)\(_3\) is generated, which leads to the decrease of oil recovery.

4. Conclusions
Comparing three different flocculants (calcium hydroxide, polyaluminium chloride and ferric chloride) to C. vulgaris, we found that the effect of aluminum salt and ferric salt is better. The inorganic polymer compound polyaluminium chloride has high recovery rate and fast precipitation. When the solution pH value is about 10, and 0.3 M polyaluminium chloride is added, the harvesting efficiency is the best, which can reach up to 90%. Ferric trichloride as flocculant, the recovery effect is better in the environment with weak solution alkalinity. When the pH value is 8 and the concentration of flocculant is 1.0 M, the recovery rate can be as high as 90%. Although the effect of calcium hydroxide on C. vulgaris is not as good as that of ferric salt and aluminum salt, but the removal of metal ions in the solution is more difficult. And the calcium hydroxide dissolved in water can be removed by way of CO\(_2\), so as to realize the recycling of culture. When calcium hydroxide flocculated C. vulgaris, it was found that the pH of solution had little effect on flocculating C. vulgaris. Adding 0.9 M of calcium hydroxide had the best flocculation effect on C. vulgaris, and the recovery rate reached 86%.

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