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Communication

Optimization of Alkaline Flocculation for Harvesting of *Scenedesmus quadricauda* #507 and *Chaetoceros muelleri* #862

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Abstract: A response surface methodology (RSM) was used to evaluate the effects of pH and microalgal biomass concentration (BC) on alkaline flocculating activity for harvesting one freshwater green algae *Scenedesmus quadricauda* #507 and one marine diatom *Chaetoceros muelleri* #862. The pH value and BC were in range of 9.0–12.0 and 0.20–2.30 g/L, respectively. Very high regression coefficient between the variables and the response indicates excellent evaluation of experimental data by second-order regressions. Optimum conditions for flocculating activity were estimated as follows: (i) pH 11.6, BC 0.54 g/L for strain #507 and (ii) pH 11.5, BC 0.42 g/L for strain #862. The maximum flocculating activity was around 94.7% and 100%, respectively. Furthermore, the addition of synthetic ocean water (SOW) to the freshwater #507 culture can increase the flocculating activity from 82.13%–88.79% in low algae concentration (0.52 g/L) and 82.92%–95.60% in high concentration (2.66 g/L).

Keywords: microalgae harvesting; flocculation; alkaline; response surface methodology; synthetic ocean water

1. Introduction

Microalgae cultivation for biofuel production have been considered as an important contributor to Greenhouse Gases mitigation and energy security due to its fast growth rates, voracious appetite for CO_2, wide adaptability, and high energy source content [1,2]. The two most important classes of microalgae in terms of abundance and lipid content are the green algae (*Chlorophyceae*) and diatoms (*Bacillariophyceae*) [3]. Lipid can be refined into biodiesel for land transportation and even for aviation use.

The concentration of microalgae achieved in the industrial application is usually between 0.3 and 0.5 g dry cell/L or 5.0 g dry cell/L at best [1,4]. Moreover, microalgae are small with a diameter of 1–30 μm. As a result, harvesting microalgae from their medium is difficult and expensive [5]. Flocculation is considered to be an effective and convenient bulk harvesting process, which reduces/neutralizes the negative surface charge of microalgal cells, allowing them to aggregate into larger lumps with an efficiency of >80% [1]. A large number of flocculants including toxic chemical such as aluminum, iron salts [6] and polyelectrolyte [3] as well as expensive biofloculants like chitosan [7] have been used.

Algae and cyanobacteria could be flocculated by high pH values [8,9]. Floc particles usually begin to form well above pH 10 and only complete at pH 11 [10]. Alkaline flocculation could be an attractive alternative because it is low-cost, low energy consumption, non-toxic to microalgal cells and the high pH effectively sterilizes the microalgal biomass as well as the process water. Previous studies have investigated the interactive effects of pH, Mg^{2+}, Ca^{2+}, concentration and microalgal biomass concentration on flocculation of *Chlorella*. The flocculation activity is highly variable and is influenced mainly by the amount of magnesium hydroxide [8,11–15]. This method was studied to a number of microalgal strains (such as *Chlorella vulgaris* [11–13], *Scenedesmus* sp. [13], *Chlorococcum* sp. [13], *Dunaliella salina* [14]). Besson and Guiraud have found the flow rate of NaOH addition had no effect on the *Dunaliella salina* recovery efficiency and non-harvested cells remained viable during pH increase which could be used as inoculum for a new culture [8].

During the practical production, the values of pH and biomass concentration (BC) are not fixed, so it not appropriate to use the one factor experimental design. As we know, few researches investigated the interacting effects of the key factors: pH value and BC to date. In this study, response surface methodology (RSM) was used to evaluate the effects of the two factors of alkaline flocculation of one freshwater green algae *Scenedesmus quadricauda* #507 and one marine diatom *Chaetoceros muelleri* #862. Furthermore, the addition of synthetic ocean water (SOW) to the medium for flocculating activity improvement was also investigated.

2. Materials and Methods

2.1. Microalgal Strain and Culture Conditions

Freshwater green algae *Scenedesmus quadricauda* #507 and marine diatom *Chaetoceros muelleri* #862 were obtained from Freshwater Algae Culture Collection (Wuhan, Hubei, China). For the cultivation of two strains, BG11 medium [16] and f/2 medium [17] were used respectively. About 100 mL pre-culture broths mentioned above were inoculated into a vertical tubular photobioreactor containing 1.0 L medium. The vertical tubular photobioreactor consisted of glass tubes of 70.0 cm heights and 5.0 cm outside diameters. Light was supplied by cool white fluorescent lamps at the single side of the photobioreactor (light intensity: 200 ± 50 μE/(m^2 s)). Aeration and mixing were achieved by the sparging air enriched with 6.0% CO_2 through a glass-filter, which was inserted to the bottom of the reactor and the flow rate of gas was 0.5 vvm regulated by the gas flow meter (Model G, Aalborg Instruments & Controls, Inc., Orange-burg, NY, USA). The temperature of the culture media was 25 ± 1 ℃ regulated by the room air conditioner (Gree Electric Appliances Inc., Zhuhai, Guangdong, China). After 6 days of cultivation, the cultures were used for flocculation experiment.

2.2. Determination of Flocculating Activity

After the flocculation of microalgal cells; an aliquot of culture was withdrawn and used to measure OD_{680} (optical density at the wavelength of 680 nm). The flocculation activity was calculated according to the following equation:

$$\textit{Flocculating activity}\ (\%) = (1 - A/B) \times 100 \quad (1)$$

where, *A* is the optical density of the sample at 680 nm and *B* is the optical density of the algal culture before the flocculation measured at 680 nm.

2.3. Alkaline Flocculation

Similar to Wu *et al.*'s method [8], flocculation experiments were run with small volumes of medium (25 mL) distributed in cylindrical glass tubes (50 mL). Effective flocculation was achieved simply by adjusting the pH accurately between 9.0 and 12.0 with 1.0 N NaOH or 1.0 N HCl. pH was measured by a portable pH analyzer (Yilun, pH-3C, Shanghai, China). After the pH had been adjusted, the glass tube was shaken thoroughly about 10 s and allowed to stand at room temperature for 30 min. Then, an aliquot of medium was withdrawn and used to measure OD_{680}.

2.4. Starving the Cultures of CO_2 Prior to Harvest

"Starving" procedure as follows: the pH of the photosynthesizing algae to rise by allowing the cells to remove excess CO_2 from the medium for 180 min prior to harvest by bubbling air through the medium.

2.5. Effect of Synthetic Ocean Water on Flocculating Activity

For the later experiment, SOW was added to the medium of freshwater green algae prior to pH adjustment. Composition of SOW: NaCl 24.540 g/L, Na_2SO_4 4.090 g/L, KCl 0.700 g/L,

$NaHCO_3$ 0.200 g/L, KBr 0.100 g/L, H_3BO_3 0.003 g/L, NaF 0.003 g/L, $MgCl_2$ $6H_2O$ 11.100 g/L, $CaCl_2$ $2H_2O$ 1.540 g/L, $SrCl_2$ $6H_2O$ 0.017 g/L. Final salinity is 35 psu. The final dilution factors of SOW were 0.001, 0.002, 0.004 and 0.006 (Dilution factors = SOW additive amount/Total amount).

2.6. Experimental Design and Statistical Analysis

A central composite design (CCD) was used for the experiments to investigate the effects of pH value and BC on the flocculating activity. Five level-2 factor experimental blocks were constructed using the Design-export 8.0 software (Stat-Ease, Minneapolis, MN, USA), and the quality of analysis model evaluated based on an analysis of variance (ANOVA). The response variable (*Y*) that representing the flocculating activity was fitted using a second-order model in the form of a quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{m} \beta_1 x_i + \sum_{i<j}^{m} \beta_{ij} x_i x_j + \sum_{i=1}^{m} \beta_{ii} x_i^2 \quad (2)$$

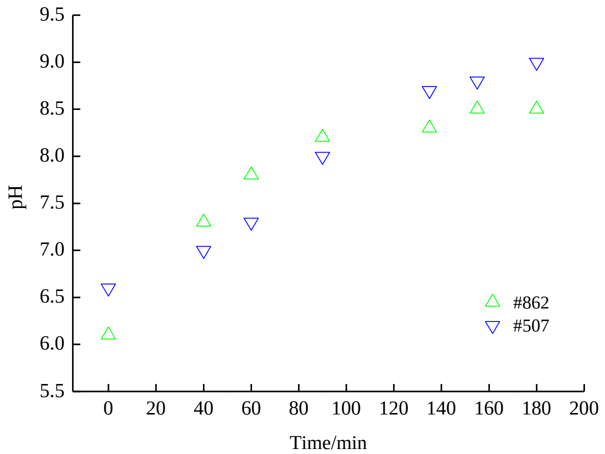
where, *Y* is the response variable to be modeled, x_i and x_j are independent variables representing the pH and BC, β_0, β_i, and β_{ii} are the offset term, linear coefficient, and quadratic coefficient, respectively, and β_{ij} is the term that reflects the interaction between x_i and x_j [18].

3. Results and Discussion

3.1. Starving the Cultures of CO_2 Prior to Harvest

The amount of flocculant needed to flocculate can affect the operating cost of algae harvesting systems. Natural increase of culture pH by photosynthesis could reduce the amount of base consumed [14]. As Figure 1 shows, because of the photosynthesizing, pH of algae cultures rose from pH 6.1 to 8.5 (*Chaetoceros muelleri* #862) and from pH 6.6 to 9.0 (*Scenedesmus quadricauda* #507) by allowing the cells to remove excess CO_2 from the medium for 3 h prior to harvest by bubbling air through the medium. By starving the cultures of CO_2 just prior to harvest, it is possible to reduce NaOH additive amount. Schlesinger *et al.* [10] reported this operation would halve the amount of $Ca(OH)_2$ needed to induce flocculation when used $Ca(OH)_2$ as a type of alkaline flocculant.

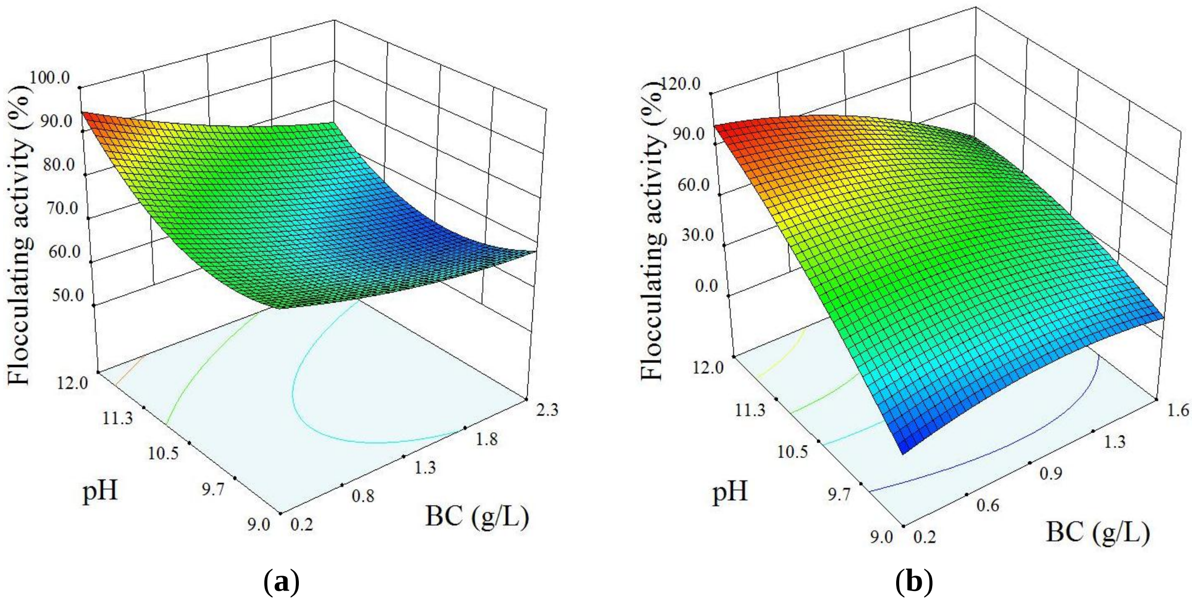
Figure 1. The change of pH values after starving the cultures of CO_2 (#507: *Scenedesmus quadricauda* #507 and #862: *Chaetoceros muelleri* #862).



3.2. Optimization of Alkaline Flocculation

In the present study, a three-dimensional plot of the response surfaces showed the interacting effects of the key factors, pH value and BC on flocculating activity (Figure 2). For the strain #507, the maximum flocculating activity was 94.7%, corresponding to pH value and BC of 11.6 and 0.54 g/L, respectively. For the strain #862, the maximum flocculating activity was 100%, corresponding to pH value and BC of 11.5 and 0.42 g/L, respectively.

Figure 2. Response surface plot representing effect of pH and biomass concentration on flocculating activity (**a**) *Scenedesmus quadricauda* #507; (**b**) *Chaetoceros muelleri* #862).



The ANOVA for the second-order regression equation (Table 1) showed that the *F*-values of the two models were 104.87 and 73.46, respectively, which implied the two models were both significant. Regression analysis of the experimental design demonstrated that the linear model terms (BC, pH), quadratic model terms (BC^2, pH^2) and interactive model terms (BC × pH) were all highly significant ($p < 0.05$). Table 2 presents the estimated regression coefficients for all factors and their respective values with regards to the flocculating activity. As shown in Table 2, compared to the coefficient estimate, it clearly appears that the significance follows: pH^2 > BC > pH > BC × pH > BC^2 for *Scenedesmus quadricauda* #507 and pH > BC × pH > BC > BC^2 > pH^2 for *Chaetoceros muelleri* #862. The positive and negative coefficients for the linear terms suggested the improved flocculating activity can be achieved by increasing pH value or decreasing BC. The final equations in terms of the actual factors are shown in Table 3. The pred-R^2 of 0.915 and 0.912 were in reasonable agreement with the adj-R^2. The proposed model equations provide satisfactory and accurate results.

Table 1. Analysis of Variance (ANOVA) for Response Surface Quadratic Model.

Source	Sum of Squares	DF [a]	Mean Square	*F*-Value	*p*-value
Scenedesmus quadricauda #507					
Model	1,207.78	5	241.56	104.87	<0.0001
BC	414.70	1	414.70	180.04	<0.0001
pH	268.41	1	268.41	116.53	<0.0001
BC × pH	26.11	1	26.11	11.34	0.0120
BC^2	26.38	1	26.38	11.45	0.0117
pH^2	493.46	1	493.46	214.23	<0.0001
Residual	16.12	7	2.30	–	–
Lack of Fit	14.24	3	4.75	10.10	0.0245
Pure Error	1.88	4	0.47	–	–
Cor Total	1,223.90	12	–	–	–
Chaetoceros muelleri #862					
Model	11,517.07	5	2,303.41	73.46	<0.0001
BC	1,423.51	1	1,423.51	45.40	0.0003
pH	7,940.07	1	7,940.07	253.23	<0.0001
BC × pH	1,216.27	1	1,216.27	38.79	0.0004
BC^2	600.57	1	600.57	19.15	0.0032
pH^2	457.43	1	457.43	14.59	0.0065
Residual	219.48	7	31.35	–	–
Lack of Fit	125.43	3	41.81	1.78	0.2903
Pure Error	94.05	4	23.51	–	–
Cor Total	11,736.55	12	–	–	–

[a] DF: degree of freedom.

Table 2. Estimated regression coefficients for flocculating activity.

Factor	Coefficient Estimate	DF [a]	Standard Error	95% CI [b] Low	95% CI High	VIF [c]
Scenedesmus quadricauda #507						
Intercept	68.94	1	0.68	67.33	70.54	–
BC	−7.20	1	0.54	−8.47	−5.93	1.00
pH	5.79	1	0.54	4.52	7.06	1.00
BC × pH	−2.56	1	0.76	−4.35	−0.76	1.00
BC^2	1.95	1	0.58	0.59	3.31	1.02
pH^2	8.42	1	0.58	7.06	9.78	1.02
Chaetoceros muelleri #862						
Intercept	57.50	1	2.50	51.58	63.42	–
BC	−13.34	1	1.98	−18.02	−8.66	1.00
pH	31.50	1	1.98	26.82	36.19	1.00
BC × pH	−17.44	1	2.80	−24.06	−10.82	1.00
BC^2	−9.29	1	2.12	−14.31	−4.27	1.02
pH^2	−8.11	1	2.12	−13.13	−3.09	1.02

[a] DF: degree of freedom. [b] CI: confidence interval. [c] VIF: variance inflation factor.

Table 3. The final equation in terms of the actual factors.

Strains	Equations	R^2	Adj-R^2	Pred-R^2
#507 [a]	$Y = +811.5 + 15.5 \times BC - 147.6 \times pH - 3.3 \times BC \times pH + 3.6 \times BC^2 + 7.5 \times pH^2$	0.987	0.977	0.915
#862 [b]	$Y = -1351.3 + 370.6 \times BC + 209.4 \times pH - 31.8 \times BC \times pH - 34.9 \times BC^2 - 7.2 \times pH^2$	0.981	0.968	0.912

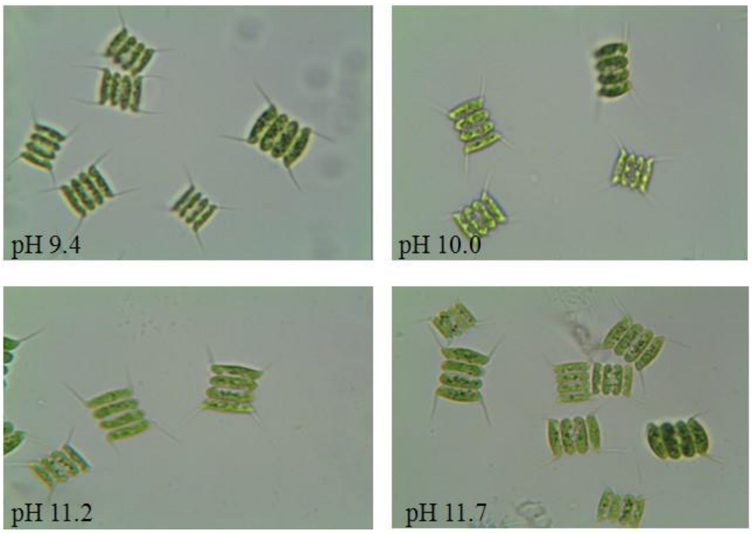
[a] *Scenedesmus quadricauda* #507; [b] *Chaetoceros muelleri* #862.

3.3. Microscopic Observation of Algal Cells with Increasing pH

As Figure 3 shows, for the strain #507, with the increasing pH values, the cells still existed integrally as normal cells, with seta disappearance vaguely. No clearly sediment produced. To the contrary, for strain #862, as the pH was increased to 10.7, substantial sediment occurred. By pH 11.8, bulk precipitation was significantly generated, the dense algal cells were wrapped in it. Blanchemain *et al.* [19] noted that cell lysis occurred after 1 h. We did not observe apparent deterioration of microalgal biomass harvested even using pH 11.5 after 6 h flocculation.

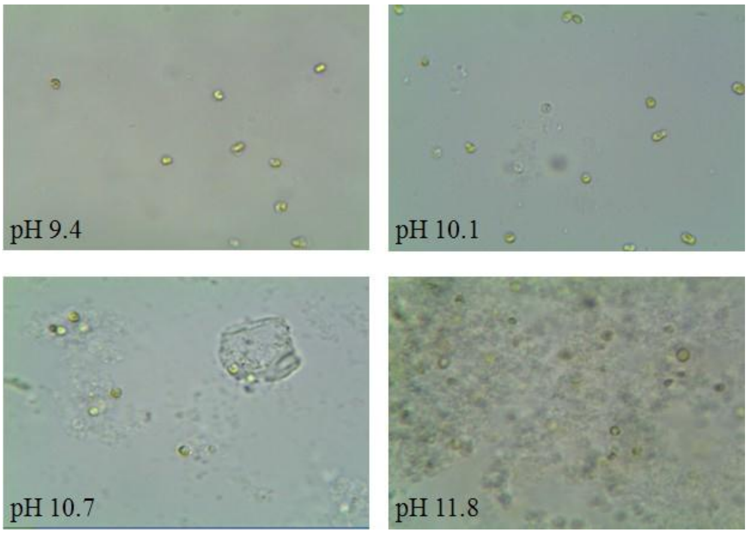
As we know, there has a high concentration Mg^{2+}, Ca^{2+} in the sea water which was precipitated when the pH value became alkaline. The flocculation is induced by precipitation of $Mg(OH)_2$ at pH values between 9.5 and 11.5, $CaCO_3$ at pH value >9.5 [20]. Wu *et al.* [8] explained that $Mg(OH)_2$ precipitate coagulated microalgal cells by sweeping flocculation and charge neutralization. However, Schlesinger *et al.* [10] found flocculation is not related to co-precipitation with iron, magnesium, phosphate or $Ca(OH)_2$. Flocculation is still probably related to cell characteristics.

Figure 3. Morphotogical changes of algal cells with increasing pH observed with polarizing microscope at ×400 magnification after 6 h flocculation (**a**) *Scenedesmus quadricauda* #507; (**b**) *Chaetoceros muelleri* #862.



(**a**)

Figure 3. *Cont.*

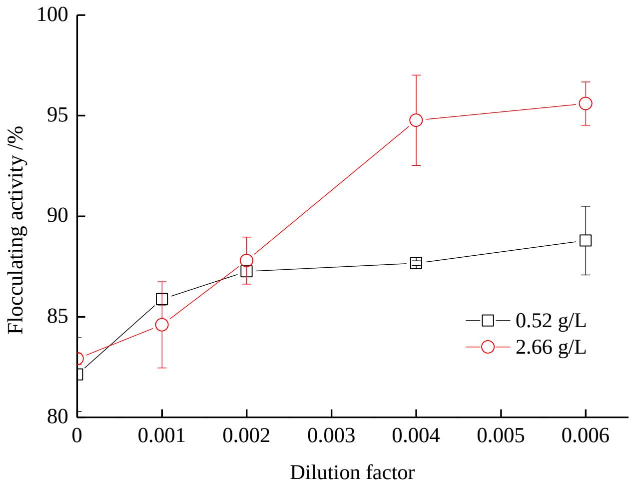


(**b**)

3.4. Improvement of Flocculating Activity with the Addition of Synthetic Ocean Water

As Figure 4 shows, a cheap additive, SOW, was added to the freshwater *Scenedesmus quadricauda* #507 culture can improve flocculating activity obviously. The flocculating activity can be increased from 82.13%–88.79% in low algal concentration (0.52 g/L), and 82.92%–95.60% in high algal concentration (2.66 g/L). In low algal concentration condition, a small quantity of SOW (dilution factor is 0.002) is needed to close to the max flocculating activity, while a large quantity of SOW (dilution factor is 0.006) is needed to close to the max flocculating activity in the high concentration condition. The two microalgae concentrations' ratio is about 5, but the quantity ratio of SOW needed is 3. The result means the denser the cell suspension, the less flocculant needed per cell. No direct linear relationship between number of cells to be flocculated and the amount of flocculant required. It was similar with Schlesinger *et al.*'s [10] result whereby they found the amount of flocculant required is directly related to the logarithm of cell density.

Figure 4. Improvement of flocculating activity of *Scenedesmus quadricauda* #507 by synthetic ocean water added (pH 11.5, 10 min stand of the concentration 0.52 g/L).



4. Conclusions

Alkaline flocculation is a potentially useful method for microalgae bulk harvesting. The analysis from the response surface (RMS) methodology emphasized that pH, BC and the interaction between the two factors all impact the alkaline flocculation process. The cell damage mechanism on high pH value conditions would be studied further. Like a rich and available resource of seawater, SOW addition to the freshwater algae culture can improve flocculating activity. The denser BC, the less SOW needed per unit of biomass amount.

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Author Contributions

Shuhao Huo wrote the main part of the paper and performed the experiments. Zhongming Wang, Shunni Zhu, Renjie Dong, Zhenhong Yuan revised the paper. Other authors read and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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