Exploration on Bioflocculation of *Nannochloropsis oculata* Using Response Surface Methodology for Biodiesel Production

Duraiarasan Surendhiran and Mani Vijay

Bioelectrochemical Laboratory, Department of Chemical Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Tamilnadu 608002, India

Correspondence should be addressed to Mani Vijay; drmvijay2009@gmail.com

Received 31 August 2013; Accepted 19 November 2013; Published 5 February 2014

Academic Editors: A. W. Gertler, R. Leyva, and R. Pedicini

Copyright © 2014 D. Surendhiran and M. Vijay. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Harvesting of algal biomass in biodiesel production involves high energy input and cost incurred process. In order to overcome these problems, bioflocculation process was employed and the efficiency of this process was further improved by the addition of a cationic inducer. In this work marine *Bacillus subtilis* was used for bioflocculation of *Nannochloropsis oculata* and ZnCl₂ as cationic inducer. This study worked under the principle of divalent cationic bridging (DCB) theory. Under temperature stress and high pH, the bacterium produced exopolysaccharide that bound with microalga *Nannochloropsis oculata* and flocculated them. A maximum efficiency of 95.43% was observed with the optimised RSM parameters—temperature 30.78°C, pH 10.8, flocculation time 6.7 h, bioflocculant size 0.38 mL, and cationic inducer concentration 0.035 mM. The present investigation focused on the cost effective harvesting of microalga on a larger scale for biodiesel production than using toxic, ecofriendly chemical flocculants.

1. Introduction

The world’s oil production is expected to decline in next ten decades due to burgeoning population and uncontrolled urbanization that have created serious problems of energy requirement. Global warming is one of the major environmental problems occurring because of increasing CO₂ concentration in the atmosphere due to excessive consumption of fossil fuels [1]. Thus an alternate fuel has to be generated against fossil fuel. Biodiesel, also known as fatty acid methyl esters (FAMES), is a potential substitute for conventional diesel fuel, which is obtained by the transesterification of triglyceride with a short chain alcohol (methanol or ethanol) [2–6]. In addition, the biodiesel has more advantages over diesel fuel because of its renewability, biodegradability, and lower emission of CO₂ [5]. Biofuels produced from crops have become a major controversy due to food versus fuel competition and animal fat cannot be considered as a continuous supply of feed stock [7], whereas algae can be grown using poor quality waters as they do not compete with food crops for arable land and water [8]. Moreover, microalgae have advantages of high growth rate and contain more amounts of lipids from 20% to 80% of dry cell weight than the conventional oil crops which produce only 5% of dry weight [9].

Considering the growing demand for energy, algae are one of the most important energy sources for future [10], because oil crops, waste cooking oils, and fats cannot meet current and future demand for biodiesel [11]. Thus, microalgae represent one of the viable and renewable sources of biodiesel feedstock that can meet global demand for transport fuels [11, 12]. Intensive cultivation for production of large quantities of microalgal biomass requires a proper harvesting technique. One of the major problems in large scale productions of microalgae is the development of efficient separation of cells from culture broth and also to maintain their viability and bioactivity prior to use in the field [13].

Because of the small size of the algal cells (3–30 μm in diameter) [14], biomass harvesting in microalgae represents one of the significant cost factors in the production of biodiesel from microalgae [15–17]. Therefore, microalgae harvesting process became a challenging task and commercial production of biodiesel from microalgae is economically unfeasible. Different studies showed that the harvesting cost...
of algal production in open ponds accounts for more than 20–30% of the total cost of biodiesel production [18]. The potential of microalgae for biodiesel production is based on the microalgal biomass concentrate [14]. Thus, to minimize the energy consumption of harvesting microalgae, an integrated approach is needed [19]. Therefore, microalgae harvesting is one of the difficult processes thus obstructing the development of algae biodiesel.

A significant reduction in the cost of microalgal biomass production will require cost-efficient methods for harvesting microalgae [20]. Many separation methods such as centrifugation, gravity sedimentation, (ultra)filtration, and ultra sound waves have been developed for microalgae recovery. However, each has its disadvantages that affect the overall economics of the process. Centrifugation requires high energy input and initial capital cost and the process involves exposing cells to high gravitational and shear forces which damage the cell structure. Second, the processing of large culture volumes can be time-consuming. Filtration and screening require regular replacement of filters, screens, and membranes and can be very time consuming. Gravity sedimentation is a slow process and electroflootation requires replacement of worn electrodes that have high cost of electricity consumption [21].

Evaluation of several harvesting methods showed that flocculation is the most promising cost and energy efficient alternative [22, 23]. During flocculation, the dispersed microalgal cells aggregate and form flocs with higher sedimentation rate [24, 25]. In addition, it allows the handling of large volumes of cultures and cells harvested by flocculation are in better physical condition [26].

Chemical substances that are commonly used as flocculants are highly toxic to humans and nondegradable and the intermediate byproducts of degradation are also harmful to the ecosystem [16, 27, 28]. Now researches are being focused on bioflocculation agent that is advantageous over chemical flocculant due to their biodegrading nature, high efficiency, nontoxicity, and ecofriendliness [29–32]. Bioflocculants are primarily made up of polysaccharides secreted by microorganisms extracellularly. These exopolymeric substances orexopolysaccharides (EPS) are generally produced by bacteria, yeast, and fungi during their growth [33], playing a vital role in a flocculation process.

EPS produced by the bacterial culture is lesser and would be insufficient when harvesting in large scale; that is, the extracellular product from the bacterial cell is cost consuming. To overcome such a problem, we used the whole live culture as bioflocculant for harvesting Nannochloropsis oculata for biodiesel production. To increase the efficiency of bioflocculation, the divalent cations had been added as an inducer in the bioflocculation process to neutralize the similar net negative charges of EPS and the microalgal cell wall.

As to the best of our knowledge, there are scanty reports available on whole culture as bioflocculant, thus this would be one of the first reports on bioflocculation using live cells. The present investigation involved the bioflocculation process enhanced by inducer which was selected through cell viability test and optimized using Response Surface Methodology (RSM) with important physical parameters like temperature, pH, flocculation time, bioflocculant size, and cationic inducer concentration.

2. Materials and Methods

2.1. Organism and Culture Medium. Nannochloropsis oculata, obtained from the Central Marine and Fisheries Research Institute (CMFRI), Tuticorin, Tamilnadu (India), was grown in sterile Walne’s medium. The filtered sterilized sea water was enriched with required quantity of Walne’s medium composition containing (g L^{-1}): NaNO_3, 100; Na_2HPO_4 \cdot 2H_2O, 20.0; Na_2EDTA, 4.0; H_3BO_3, 33.6; MnCl_2 \cdot 4H_2O, 0.36; FeCl_3 \cdot 6H_2O, 13.0; vitamin B_12, 0.001 and vitamin B_1, 0.02. The trace metal solution contained (g L^{-1}): ZnSO_4 \cdot 7H_2O, 4.4; CoCl_2 \cdot 6H_2O, 2.0; (NH_4)_6Mo_7O_{24} \cdot 4H_2O, 0.9; and CuSO_4 \cdot 5H_2O, 2.0. The medium was adjusted to pH 8 and autoclaved at 121°C for 20 min. The filter sterilized vitamins were added after cooling. The contents were later introduced into a 250 mL Erlenmeyer flask and finally transferred to 25 L photobioreactor (PBR). Mixing was provided by sparging air from the bottom of the PBR; lighting was supplied by cool-white fluorescent tubes with an intensity of 5000 lux. End of the log phase culture was used for the coagulation experiments.

2.2. Culture for Bioflocculation. The marine bacterial culture Bacillus subtilis (MTCC 10619) was used as the bioflocculant, obtained from the Department of Marine Biology, Parangipettai, Annamalai University, India. The bacterial culture was cultivated for growth and bioflocculant production using nutrient broth supplemented with 3% NaCl subcultured periodically and stored as stocks on nutrient agar slants at 4°C.

2.3. Evaluation of Bioflocculation Experiment: One-Factor-at-a-Time Design. Flocculation experiments were carried out in stationary growth phase of microalgae. A quantity of 50 mL of Nannochloropsis oculata was used for optimization study. The effects of bioflocculation parameters, namely, temperature, pH, time, bioflocculant concentration, and cationic inducer size, were individually experimented by analyzing flocculation efficiency. For the effect of pH, the culture was divided in a series of test tubes, and the pH was adjusted to fixed values by the addition of 1 M HCl or 1 M NaOH, ranging from approximately 6.0 to 10. Likewise, for effect of temperature the test tubes were incubated at desired temperatures. After the parameter setup, each tube was kept in orbital shaker (Model-Technico, Honeywell Ltd, India) and stirring speed was maintained at 250 rpm. The initial microalgal biomass concentration in the tubes was estimated from the optical density of 750 nm (OD_{750}) in UV-VIS Spectrophotometer (Model-SL159, ELICO Ltd, India). At the end of the bioflocculation time, the optical density of the supernatant was measured at half the height of the clarified culture. Culture broth containing no bioflocculant was used.
Bioflocculation efficiency was calculated by the following [34, 35]:

\[
\text{Flocculation Efficiency} (\%) = \left(1 - \frac{A}{B}\right) \times 100, \quad (1)
\]

where \( A = \text{OD}_{750} \) value of sample and \( B = \text{OD}_{750} \) value of control.

### 2.4. Response Surface Methodology: CCD

A central composite design (CCD) of the experiments was formulated to investigate five flocculation parameters. Each 50 mL culture of *Nannochloropsis oculata* was added into test tubes and the parameters were set according to the orthogonal values of central composite design (CCD) (Table I). RSM is known to evaluate the interaction between the significant factors of an experiment and optimize them [23]. Five-level factor experiment setup was designed using Design Expert Software version 8.0.7.1, Stat-Ease, Minneapolis, USA, and the quality of analysis model was based on an analysis of variance (ANOVA). The response variable \( Y \), representing the bioflocculation activity, was fitted using a second-order polynomial equation given as

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_4 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5 + \beta_{11} X_1^2 + \beta_{12} X_2^2 + \beta_{13} X_3^2 + \beta_{14} X_4^2 + \beta_{15} X_5^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{55} X_5^2 \quad (2)
\]

where \( Y \) is the predicted response, \( \beta_0 \) was the constant, \( X_1- X_5 \) were the input variables, \( \beta_1- \beta_5 \) were the linear coefficients, \( \beta_{12}- \beta_{45} \) were the second order interactive coefficients, and \( \beta_{11}- \beta_{55} \) were the quadratic coefficients.

The actual value of coded levels of different parameters which are temperature \( X_1 \), pH \( X_2 \), flocculation time \( X_3 \), bioflocculant size \( X_4 \), and cationic inducer concentration \( X_5 \) is presented in Table I and its influence on harvesting of microalgae by flocculation, represented as \( Y \), the response variable, has been investigated. The actual values of coded level “0” were fixed based on one-factor-at-a-time method.

### 3. Results and Discussion

#### 3.1. Variables Influencing the Bioflocculation Process

##### 3.1.1. Effect of Temperature on Bioflocculation

The flocculation efficiency reached its maximum as the temperature was increased till 30°C, after which the flocculation efficiency decreased (Figure 2). Effective process occurred at a temperature of 30°C, as the cells of marine bacterium, *B. subtilis* (MTCC 10619), were able to produce more bioflocculant, that is, exopolysaccharide (EPS) at high pH stress condition. A rapid decrease in efficiency was observed, when the temperature was raised beyond 30°C, which was due to the susceptibility of microalgal cells as well as molecular mobility at higher temperature. Thus collision occurred between bioflocculant and microalgal cells, which lead to cell distortion [36]. Moreover, as the microalgae and the bioflocculant producing bacteria are from marine sources, supplementation of additional medium components/nutrients may not be necessary.

##### 3.1.2. Effect of pH on Bioflocculation

pH is one of the most important factors for harvesting microalgae; hence the influence of pH on bioflocculation efficiency was tested with a pH range from 6 to 10. From the statistical experimental results, the effect of pH on flocculation efficiency was highly significant \((P < 0.01)\) and the flocculation efficiency was found to be higher with increase in pH, that is, 10. This result is in agreement with previous studies [20]. As pH increases, the negative charge of microalgal cells increases. This phenomenon could be a major cause for flocculation by higher pH. This is due to difference in protonation conformational changes and structural alterations in flocs.

##### 3.1.3. Effect of Bioflocculant Size on Bioflocculation

As the time prolonged, deterioration was observed in bioflocculation efficiency. Bioflocculants (EPS) are generally found to be produced during late exponential phase or stationary phase of the bacterial growth (Figure 1) [22], after which the concentration or the production of the polymer remains constant in the medium. Hence as the time increased beyond the production time, flocculation decreased. Lower efficiency

---

**Table I: Coded values based on the factor at a time experiment for the 5 variables employed in the study.**

| Code | Variables                  | -2 | -1 | 0  | +1 | +2 |
|------|----------------------------|----|----|----|----|----|
| \(X_1\) | Temperature (°C)         | 20 | 25 | 30 | 35 | 40 |
| \(X_2\) | pH                       | 6  | 7  | 8  | 9  | 10 |
| \(X_3\) | Flocculation time (hr)   | 2  | 4  | 6  | 8  | 10 |
| \(X_4\) | Bioflocculant size (mL)  | 0.1| 0.2| 0.3| 0.4| 0.5|
| \(X_5\) | Cationic inducer concentration (mM) | 0.01| 0.02| 0.03| 0.04| 0.05|

---

**Figure 1:** Time course of batch culture of *Bacillus subtilis* MTCC10619. Blue triangles: cell dry weight (g/L) and red bullets: bioflocculant production (g/L).
Figure 2: Continued.
using central composite design (CCD) with 50 runs and 7 central points. The predicted and experimental responses from each experiment were tabulated (Table 2). A positive sign denoted that the effect of the variables on flocculation was greater at a higher concentration whereas a negative symbol represented that influence of variable on flocculation is greater at a lower concentration.

Multiple linear regression analysis was carried out using a second-order polynomial equation that was fitted to the above data as

\[ Y_{\text{biofloc}} = 96.5512 + 3.65938X_1 + 1.33237X_2 + 1.15548X_3 - 1.33237X_4 + 0.58843X_5 - 0.20093X_6 + 0.37406X_7 - 0.45406X_8 - 0.271563X_9 \]

where \( Y_{\text{biofloc}} \) is the response variable, \( X_1 \) to \( X_9 \) are the linear effects of the independent variables such as temperature, pH, flocculation time, bioflocculant size, and cationic inducer size, respectively, \( X_1 \), \( X_2 \) to \( X_4 \), \( X_5 \) are the interactive terms of the variables, and \( X_1^2 \) to \( X_8^2 \) are squared effects of the variables.

The variation of different parameters which are temperature \( (X_1) \), pH \( (X_2) \), flocculation time \( (X_3) \), bioflocculant size \( (X_4) \), and cationic inducer concentration \( (X_5) \) is presented in Table 1 and its influence on harvesting of microalgae, which represents response variable \( (Y) \), has been investigated.

The goodness of fit of regression equation developed could be measured by determination coefficient. The \( R^2 \) value of 0.8648 and adjusted \( R^2 \) of 0.7715 showed that the model could be significant predicting the response and explaining 95% of the variability in the data. Table 4 revealed the

![Figure 2: 3D Response surface and contour plots representing various interactive effects of variables on bioflocculation.](image)
Table 2: Central composite design matrix of orthogonal values with observed responses on bioflocculation efficiency.

| Run | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | Bioflocculation efficiency (%) |
|-----|-------|-------|-------|-------|-------|-------------------------------|
|     |       |       |       |       |       | Experimental value | Predicted value |
| 1   | 35    | 9     | 8     | 0.2   | 0.02  | 93.43            | 94.36            |
| 2   | 25    | 7     | 8     | 0.4   | 0.02  | 85.39            | 81.56            |
| 3   | 35    | 7     | 8     | 0.2   | 0.04  | 92.79            | 89.16            |
| 4   | 35    | 9     | 4     | 0.4   | 0.04  | 91.33            | 94.46            |
| 5   | 25    | 7     | 8     | 0.2   | 0.04  | 88.78            | 87.55            |
| 6   | 25    | 7     | 8     | 0.4   | 0.04  | 90.00            | 82.51            |
| 7   | 35    | 7     | 8     | 0.4   | 0.02  | 83.81            | 86.53            |
| 8   | 35    | 7     | 4     | 0.2   | 0.02  | 79.33            | 82.25            |
| 9   | 30    | 8     | 6     | 0.5   | 0.03  | 94.02            | 92.80            |
| 10  | 30    | 8     | 6     | 0.3   | 0.03  | 95.43            | 95.00            |
| 11  | 30    | 8     | 2     | 0.3   | 0.03  | 83.11            | 83.69            |
| 12  | 35    | 9     | 4     | 0.4   | 0.02  | 92.83            | 93.95            |
| 13  | 25    | 7     | 8     | 0.2   | 0.02  | 76.47            | 76.10            |
| 14  | 35    | 7     | 8     | 0.2   | 0.02  | 81.45            | 85.91            |
| 15  | 25    | 9     | 4     | 0.4   | 0.02  | 85.44            | 84.18            |
| 16  | 40    | 8     | 6     | 0.3   | 0.03  | 92.08            | 88.55            |
| 17  | 30    | 8     | 6     | 0.1   | 0.03  | 90.41            | 86.35            |
| 18  | 25    | 7     | 4     | 0.4   | 0.04  | 83.12            | 80.84            |
| 19  | 30    | 8     | 10    | 0.3   | 0.03  | 94.10            | 88.23            |
| 20  | 25    | 7     | 4     | 0.2   | 0.02  | 74.11            | 69.71            |
| 21  | 35    | 9     | 8     | 0.4   | 0.02  | 94.34            | 93.43            |
| 22  | 35    | 9     | 4     | 0.2   | 0.04  | 94.22            | 94.50            |
| 23  | 35    | 9     | 4     | 0.2   | 0.02  | 92.66            | 92.22            |
| 24  | 25    | 9     | 4     | 0.4   | 0.04  | 85.21            | 84.16            |
| 25  | 30    | 10    | 6     | 0.3   | 0.03  | 92.42            | 85.67            |
| 26  | 25    | 9     | 4     | 0.2   | 0.04  | 77.23            | 79.36            |
| 27  | 25    | 9     | 8     | 0.4   | 0.04  | 83.90            | 84.32            |
| 28  | 25    | 9     | 8     | 0.4   | 0.02  | 79.54            | 86.39            |
| 29  | 25    | 9     | 8     | 0.2   | 0.02  | 80.12            | 82.49            |
| 30  | 25    | 9     | 8     | 0.2   | 0.04  | 84.98            | 82.20            |
| 31  | 25    | 9     | 8     | 0.2   | 0.04  | 74.48            | 77.84            |
| 32  | 35    | 7     | 4     | 0.4   | 0.04  | 88.79            | 89.07            |
| 33  | 35    | 7     | 4     | 0.4   | 0.02  | 86.90            | 85.55            |
| 34  | 25    | 7     | 4     | 0.2   | 0.04  | 73.02            | 74.48            |
| 35  | 35    | 9     | 8     | 0.2   | 0.04  | 87.65            | 94.59            |
| 36  | 25    | 7     | 8     | 0.2   | 0.04  | 73.75            | 78.82            |
| 37  | 30    | 6     | 6     | 0.3   | 0.03  | 70.19            | 71.66            |
| 38  | 20    | 8     | 6     | 0.3   | 0.03  | 66.39            | 64.64            |
| 39  | 30    | 8     | 6     | 0.3   | 0.05  | 92.49            | 90.62            |
| 40  | 25    | 9     | 4     | 0.2   | 0.02  | 72.99            | 77.61            |
| 41  | 35    | 7     | 8     | 0.4   | 0.04  | 88.53            | 88.01            |
| 42  | 30    | 8     | 6     | 0.3   | 0.01  | 90.20            | 86.78            |
| 43  | 35    | 9     | 8     | 0.4   | 0.04  | 89.52            | 91.89            |

Statistical significance of each coefficient. Smaller probability (P) values, that is, smaller than 0.05 (P < 0.05) and larger magnitude of "t" values indicate the significance of the model. The coefficients of this response, namely, $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, $X_1^2$, $X_2^2$, $X_3^2$, $X_4^2$, $X_1X_2$, $X_1X_3$, $X_2X_4$, $X_2X_5$, and $X_3X_4$ were found to be most significant of this model (P < 0.05).

ANOVA table illustrated (Table 3) the calculated $F$ value (9.27) and a low $P$ value ($P = 0.0001$) demonstrated that the quadratic model was highly significant.

Three dimensional response surface plots and contour plots for the bioflocculation efficiency were shown in Figure 2. The shapes of the contour plots indicate the
Table 3: ANOVA table for response surface function on bioflocculation efficiency.

| Source        | DF | SS        | MS  | F       | P       |
|---------------|----|-----------|-----|---------|---------|
| Regression    | 20 | 2725.56   | 136.28 | 9.27    | <0.0001 |
| Linear        | 5  | 1617.27   | 323.45 | 0.31    | 0.9740  |
| Square        | 10 | 127.39    | 12.74 | 13.35   | <0.0001 |
| Interaction   | 5  | 980.90    | 196.18 | 0.75    | 0.7045  |
| Residual Error| 29 | 426.21    | 14.70 |         |         |
| Total         | 49 | 3151.77   |       |         |         |

DF: degree of freedom; SS: sum of squares; MS: mean square; F: Fischer’s value; P: probability value.

Table 4: Comparison of different harvesting methods and their efficiencies.

| Method                           | Microalga                      | Harvesting efficiency (%) | References |
|----------------------------------|--------------------------------|---------------------------|------------|
| Bioflocculation with whole cell  | Nannochloropsis oculata        | >95                       | Current study |
| Flocculation with polyelectrolytes | Chaetoceros calcitrans        | >90                       | [13]        |
| Flocculation with γ-poly glutamic acid | Chlorella vulgaris and Nannochloropsis oculata | >90  | [23]   |
| Flocculation with AlCl₃         | Chlorella minutissima          | >90                       | [35]        |
| Flocculation with cationic polymer | Chlorococcum sp.               | >89                       | [36]        |
| Flocculation with chitosan      | Thalassiosira pseudonana       | 90                        | [37]        |
| Centrifugation                  | Phaeodactylum tricornutum      | 94                        | [37]        |
| Increasing pH                   | Dunaliella tertiolecta         | 90                        | [38]        |

4. Conclusions

The present study dealt with the harvesting of marine microalga, Nannochloropsis oculata, using a natural flocculant marine bacterium Bacillus subtilis. The bacteria, major producer of exopolysaccharide, influenced flocculation to a greater extent. Microbial flocculant required a very low concentration of 0.035 mM chemical flocculating agent, ZnCl₂ for the process to be enhanced and efficient. Using RSM the variables were statistically optimised which resulted in 95.43% flocculation efficiency with 0.38 mL of bioflocculant. Through the study the microbial source for flocculation was explored to be essentially a better replacement of synthetic flocculant, thereby providing a potential source for ecofriendly and cost effective large scale harvesting of microalga for biodiesel production.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] H. Sakuragi, K. Kuroda, and M. Ueda, “Molecular breeding of advanced microorganisms for biofuel production,” Journal of Biomedicine and Biotechnology, vol. 2011, Article ID 410931, 11 pages, 2011.
D. G. Tao and J. Salihon, “The Optimisation of levels of the variables pH and Concentration of Ferric chloride for Harvesting marine microalgae by flocculation,” in Proceedings of the International Conference on Food Engineering and Biotechnology (IPCBEE), IACSIT Press, Singapore, 2011.

C. M. L. L. Teixeira, F. V. Kirsten, and P. C. N. Teixeira, “Evaluation of Moringa oleifera seed flour as a flocculating agent for potential biodiesel producer microalgae,” Journal of Applied Phycology, vol. 24, no. 3, pp. 557–563, 2012.

S. Salim, R. Bosma, M. H. Vermuë, and R. H. Wijffels, “Harvesting of microalgae by bio-flocculation,” Journal of Applied Phycology, vol. 23, no. 5, pp. 849–855, 2011.

W. Zhou, Y. Cheng, Y. Li et al., “Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment,” Applied Biochemistry and Biotechnology, vol. 167, no. 2, pp. 214–228, 2012.

D. Vandamme, I. Foubert, I. Fraeye, B. Meesschaert, and K. Muylaert, “Flocculation of Chlorella vulgaris induced by high pH: role of magnesium and calcium and practical implications,” Bioresource Technology, vol. 105, pp. 114–119, 2012.

D. Surendhiran and M. Vijay, “Study on flocculation efficiency for harvesting Nannochloropsis oculata for biodiesel production,” International Journal of ChemTech Research, vol. 5, no. 4, pp. 1761–1769, 2013.

D. G. Kim, H. J. La, C. Y. Ahn, Y. H. Park, and H. M. Oh, “Harvest of Scenedesmus sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures,” Bioresource Technology, vol. 102, no. 3, pp. 3163–3168, 2011.

H. Zheng, Z. Gao, J. Yin, X. Tang, X. Ji, and H. Huang, “Harvesting of microalgae by flocculation with poly(γ-glutamic acid),” Bioresource Technology, vol. 112, pp. 212–220, 2012.

R. M. Knuckey, M. R. Brown, R. Robert, and D. M. F. Brampton, “Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds,” Aquacultural Engineering, vol. 35, no. 3, pp. 300–313, 2006.

Z. Yang, Y. Shang, Y. Lu et al., “Flocculation properties of biodegradable amphoteric chitosan-based flocculants,” Chemical Engineering Journal, vol. 172, no. 1, pp. 287–295, 2011.

L. Borré, J. A. Morón-Villarreyes, M. G. M. D'Oca, and P. C. Abreu, “Effects of flocculants on lipid extraction and fatty acid composition of the microalgae Nannochloropsis oculata and Thalassiosira weissflogii,” Biomass and Bioenergy, vol. 35, no. 10, pp. 4449–4454, 2011.

H. Yokoi, T. Arima, J. Hirose, S. Hayashi, and Y. Takasaki, “Flocculation properties of poly(γ-glutamic acid) produced by Bacillus subtilis,” Journal of Fermentation and Bioengineering, vol. 82, no. 1, pp. 84–87, 1996.

C. G. Kumar, H. S. Joo, R. Kavali, J. W. Choi, and C. S. Chang, “Characterization of an extracellular biopolymer flocculant from a haloalkalophilic Bacillus isolate,” World Journal of Microbiology and Biotechnology, vol. 20, no. 8, pp. 837–843, 2004.

H. Salehizadeh and S. A. Shojaosadati, “Extracellular biopolymeric flocculants: recent trends and biotechnological importance,” Biotechnology Advances, vol. 19, no. 5, pp. 371–383, 2001.

Y. Zheng, Z. Ye, X. L. Fang, Y. H. Li, and W. M. Cai, “Production and characteristics of a bioflocculant produced by Bacillus sp. F19,” Bioresource Technology, vol. 99, no. 16, pp. 7686–7691, 2008.

Y. Pan, B. Shi, and Y. Zhang, “Research on flocculation property of bioflocculant PG.a21 Ca,” Modern Applied Sciences, vol. 3, no. 6, pp. 106–112, 2009.

L. Gouveia and A. C. Oliveira, “Microalgae as a raw material for biofuels production,” Journal of Industrial Microbiology and Biotechnology, vol. 36, no. 2, pp. 269–274, 2009.

C. Zhu, C. Chen, L. Zhao et al., “Biofloculant produced by Chlamydomonas reinhardtii,” Journal of Applied Phycology, vol. 24, no. 5, pp. 1245–1251, 2012.
[34] H. M. Oh, S. J. Lee, M. H. Park et al., “Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49,” *Biotechnology Letters*, vol. 23, no. 15, pp. 1229–1234, 2001.

[35] A. Papazi, P. Makridis, and P. Divanach, “Harvesting *Chlorella minutissima* using cell coagulants,” *Journal of Applied Phycology*, vol. 22, no. 3, pp. 349–355, 2010.

[36] N. Uduman, Y. Qi, M. K. Danquah, and A. F. A. Hoadley, “Marine microalgae flocculation and focused beam reflectance measurement,” *Chemical Engineering Journal*, vol. 162, no. 3, pp. 935–940, 2010.

[37] M. Heasman, J. Diemar, W. O’Connor, T. Sushames, and L. Foulkes, “Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary,” *Aquaculture Research*, vol. 31, no. 8-9, pp. 637–659, 2000.

[38] J. Horiuchi, I. Ohba, K. Tada, M. Kobayashi, T. Kanno, and M. Kishimoto, “Effective cell harvesting of the halotolerant microalga *Dunaliella tertiolecta* with pH control,” *Journal of Bioscience and Bioengineering*, vol. 95, no. 4, pp. 412–415, 2003.

[39] D. C. Sobeck and M. J. Higgins, “Examination of three theories for mechanisms of cation-induced bioflocculation,” *Water Research*, vol. 36, no. 3, pp. 527–538, 2002.

[40] X. Song, X. Zhang, C. Kuang, L. Zhu, and N. Guo, “Optimization of fermentation parameters for the biomass and DHA production of *Schizochytrium limacinum* OUC88 using response surface methodology,” *Process Biochemistry*, vol. 42, no. 10, pp. 1391–1397, 2007.

[41] G. Sathyanarayanan, G. S. Kiran, and S. Joseph, “Synthesis of silver nano particles by polysaccharide bioflocculant produced from marine *Bacillus subtills*, *Colloids and Surface B,*** vol. 102, pp. 13–20, 2013.

[42] W. Raza, W. Yang, Y. Jun, F. Shakoor, Q. Huang, and Q. Shen, “Optimisation and characterisation of a polysaccharide produced by *Pseudomonas fluorescens* WR-1 and its antioxidant activity,” *Carbohydrate Polymers*, vol. 90, no. 2, pp. 921–929, 2012.