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# Flotation of blue-green algae using methylated egg ovalbumin

Hideo Maruyama*, Hideshi Seki, Akira Suzuki

*Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Minato 3-1-1, Hakodate 041-8611, Japan*

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ABSTRACT

The removal of blue-green algae by dispersed gas flotation was conducted. Methylated ovalbumin (MeOA) was used as frother and flocculant, which is a biodegradable substance. The continuous flotation experiments were conducted at different feed mass flow rate of the blue-green algae cells and MeOA. The operating variables were the mass flow rate of blue-green algae cell and MeOA, the initial concentration of the cells and MeOA, and superficial gas velocity. The results showed that the mass flow rate of MeOA was the most dominant variable affected by the removal efficiency and that the removal efficiency achieved ca. 0.85 when a ratio of the mass flow rate of MeOA and that of the cells was over 0.3. A proposed flotation model considering the adsorptions of MeOA to the cells, MeOA to bubble surface and the cells bearing with MeOA to bubble surface was applied to explain the experimental removal efficiency. The experimental and the calculated removal efficiency were within error 19%, indicating that the proposed model was valid fundamentally.



*Notation*

| | |
|---|---|
| $A$ | the cross-sectional area of bubble column [m$^2$] |
| $C_s$ | the bulk concentration of blue-green algae within the column [kg/m$^3$] |
| $C_{si}$ | the feed concentration of blue-green algae [kg/m$^3$] |
| $C_f$ | the collapsed foam liquid concentration of blue-green algae [kg/m$^3$] |
| $C_p$ | the equilibrium concentration of MeOA [kg/m$^3$] |
| $C_{pi}$ | the concentration of the feed solution for MeOA [kg/m$^3$] |
| $\varepsilon$ | gas holdup |
| $g$ | gravitational acceleration [m/s$^2$] |
| $\eta$ | the removal efficiency of blue-green algae by flotation |
| $K$ | the equilibrium adsorption constant of blue-green algae adsorbed on bubble surface [m$^3$/kg] |
| $K_b$ | the equilibrium adsorption constant of MeOA adsorbed on bubble surface [m$^3$/kg] |
| $K_s$ | the equilibrium adsorption constant of MeOA adsorbed on blue-green algae [m$^3$/kg] |
| $\mu_L$ | liquid viscosity [kg/(m s)] |
| $N$ | the number of available site for the adsorption of blue-green algae on bubble surface [kg/m$^2$] |
| $N_b$ | the saturated adsorption density of MeOA adsorbed on bubble surface [kg/m$^2$] |
| $N_m$ | the saturated adsorption density of blue-green algae on bubble surface [kg/m$^2$] |
| $N_s$ | the saturated adsorption density of MeOA adsorbed on blue-green algae [kg/kg-cell] |
| $\theta$ | the coverage fraction of bubble surface by blue-green algae |
| $\theta_b$ | the coverage fraction of MeOA on bubble surface |
| $\theta_s$ | the coverage fraction of MeOA on blue-green algae surface |
| $\rho_L$ | liquid density [kg/m$^3$] |
| $\rho_G$ | gas density [kg/m$^3$] |
| $S_b$ | the production rate of bubble surface area within the column [m$^2$/s] |
| $t$ | time [s] |
| $U_g$ | the superficial gas velocity [m/s] |
| $V$ | the bulk liquid volume within the column [m$^3$] |
| $w_{si}$ | the volumetric flow rate of the feed liquid [m$^3$/s] |
| $w_b$ | the volumetric flow rate of the brain liquid [m$^3$/s] |
| $w_f$ | the volumetric flow rate of the liquid within foam [m$^3$/s] |
| $w_{pi}$ | the volumetric flow rate of the feed MeOA solution [m$^3$/s] |
| $X$ | the adsorption density of blue-green algae on bubble surface [kg/m$^2$] |
| $X_b$ | the adsorption density of MeOA on bubble surface [kg/m$^2$] |
| $X_s$ | the adsorption density of MeOA adsorbed on blue-green algae [kg/kg-cell] |

## 1. Introduction

Blue-green algae are kinds of freshwater phytoplankton in surface water and are of concern due to the abilities to produce taste and odor compounds, which have hepatotoxic, cytotoxic and dermatotoxic behaviors, being harmful to animal and human. Therefore, the removal of blue-green algae cells has become

* Corresponding author. Tel.: +81 138 40 8813; fax: +81 138 40 8811.
*E-mail address:* maruyama@elsie.fish.hokudai.ac.jp (H. Maruyama).

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important to save healthy water environments used as water resources.

Recently the removal of these harmful blue-green algae cells has become an important process for keeping clean water environment. Several attempts for the removal of blue-green algae have been reported [1–4]. Among them, dissolved air flotation (DAF) method [5–9] has been a promising technique for more effective treatment of blue-green algae removal. The apparatus for DAF, however, has many mechanical parts and its operation is not easy. On the other hand, flotation by dispersed gas has some advantages: low energy requirements, a few mechanical parts in the apparatus, no-requirement of tedious treatments such as desorption or addition of any other chemicals and extending to a continuous operation with ease, although removal efficiency for the flotation is a bit lower than that of DAF. The authors suppose that the combination of flocculation of blue-green algae cells and flotation of its floc will be more effective.

Flocculants mostly accepted are chemically synthesized high molecular weight polymers and alum. These flocculants are environmentally undesirable, because chemically synthesized flocculants especially remain in natural environments for long period of time without degradation to less harmful form. Recently the use of extracellular polymers produced by some bacteria has been attempted to remove suspended solid as bioflocculant [10–17]. Such bioflocculants, however, have to be separated or purified from culture media by intricate treatment, which makes them expensive for practical application.

In the present study, the authors conducted flotation experiments for the removal of blue-green algae by using methylated egg ovalbumin (MeOA), which is a biodegradable surfactant proposed by our previous study [18]. The aims of the present study are clarification of the effective operating variables on the continuous flotation of blue-green algae and verification of the proposed flotation model.

## 2. Flotation model

The present model assumed the following points on the basis of observations and the preliminary experiments: (i) the volumetric flow rate of the liquid within the foam is negligibly smaller than the volumetric flow rate of the drain liquid, and (ii) the adsorption densities of MeOA on the blue-green algae and on the bubble surface are much smaller than the saturated adsorption densities on these. The detailed explanations of the present model are described as follows.

According to the mass balance about bubble dispersed phase within the column, the next relationship is obtained:

$$V\frac{dC_s}{dt} = w_{si}C_{si} - w_bC_s - w_fC_f \quad (1)$$

where $C_{si}$, $C_f$ and $C_s$ represent the initial concentration of feed solution, the bulk liquid concentration and the foamate (the collapsed foam liquid) concentration for blue-green algae, respectively. $w_{si}$, $w_b$ and $w_f$ represent the volumetric flow rate of the feed, the drain and liquid within the foam, respectively. $V$ is the volume of the liquid within the column. The term, $w_fC_f$, in the right hand side of Eq. (1) corresponds to the overhead mass flow rate of blue-green algae and can be expressed as:

$$w_fC_f = w_fC_s + S_bX \quad (2)$$

where $S_b$ and $X$ represent the production rate of bubble surface area and adsorption density of blue-green algae on bubble surface, respectively. By substituting Eq. (2) into Eq (1), Eq. (1) is rewritten as:

$$V\frac{dC_s}{dt} = w_{si}C_{si} - (w_b + w_f)C_s - S_bX \quad (3)$$

Judging from the preliminary experiments, the value of $w_f$ was regarded as smaller value than that of $w_b$. Then, Eq. (3) can be expressed approximately as:

$$V\frac{dC_s}{dt} = w_{si}C_{si} - w_bC_s - S_bX \quad (4)$$

From Eq. (4), the following relationship in the steady state within the column liquid can be obtained as:

$$w_{si}C_{si} = w_bC_s + S_bX \quad (5)$$

The removal efficiency, $\eta$, of blue-green algae is defined as:

$$\eta \equiv \frac{w_{si}C_{si} - w_bC_s}{w_{si}C_{si}} \quad (6)$$

The adsorption density, $X$, of blue-green algae can be expressed as:

$$X = N\theta = \frac{NKC_s}{1 + KC_s} \quad (7)$$

where $K$, $N$ and $\theta$ represent the equilibrium adsorption constant, the number of available site for the adsorption of blue-green algae onto bubble surface and the coverage fraction of bubble surface by blue-green algae, respectively. The authors employed Langmuir adsorption isotherm as the adsorption equilibrium relationship. $N$ can be expressed as:

$$N = N_m\theta_b \quad (8)$$

where $N_m$ and $\theta_b$ are the saturated adsorption densities of blue-green algae and the coverage fraction of MeOA adsorbed on bubble surface, respectively. Moreover, adsorption density, $X_b$, of MeOA on bubble surface and $\theta_b$ can be expressed as:

$$X_b = \frac{N_bK_bC_p}{1 + K_bC_p} \quad (9)$$

$$\theta_b \equiv \frac{X_b}{N_b} = \frac{K_bC_p}{1 + K_bC_p} \quad (10)$$

where $C_p$, $K_b$ and $N_b$ represent the equilibrium adsorption concentration, the equilibrium adsorption constant and the saturated adsorption density of MeOA, respectively. MeOA molecule can also adsorb onto blue-green algae surface, then, the adsorption equilibrium relationship can be expressed as follows:

$$X_s = \frac{N_sK_sC_p}{1 + K_sC_p} \quad (11)$$

$$\theta_s \equiv \frac{X_s}{N_s} = \frac{K_sC_p}{1 + K_sC_p} \quad (12)$$

where $K_s$, $N_s$ $X_s$ and $\theta_s$ represent the equilibrium adsorption constant, the saturated adsorption density, the adsorption density of MeOA on blue-green algae and the coverage fraction of MeOA adsorbed on blue-green algae surface, respectively. In the present system, all, MeOA, blue-green algae cells and bubble surface are present in the same liquid. Therefore, the equilibrium concentrations of MeOA involved in both the adsorption of MeOA on cells surface and that on bubble surface cannot be classified. In Eqs. (9) and (11), Langmuir adsorption isotherm is employed for adsorption equilibrium relationship between MeOA and bubble surface, and, MeOA and blue-green algae surface. In the present conditions, $X_s$ and $X_b$ can be assumed to be much smaller than $N_s$ and $N_b$, respectively. This assumption makes Eqs. (9) and (11) simply as follows:

$$X_s = N_sK_sC_p \quad (13)$$

$$X_b = N_bK_bC_p \quad (14)$$

On the other hand, at the steady state, the mass balance of MeOA within the column can be expressed as:

$$w_{pi}C_{pi} = w_bC_p + w_{si}C_{si}X_s + S_bX_b \quad (15)$$

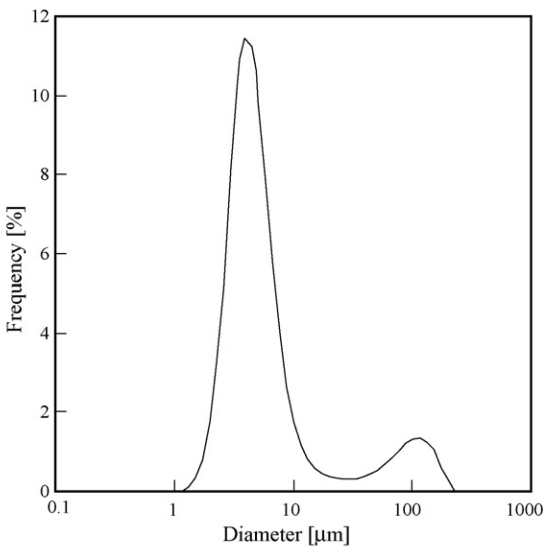


**Fig. 1.** Size distribution of blue-green algae cells used in the flotation experiments.

$C_{pi}$ and $w_{pi}$ represent the concentration of MeOA feed solution and the volumetric flow rate of MeOA feed solution, respectively. By substituting $X_s$ and $X_b$ expressed as Eqs. (13) and (14) into Eq. (15), $C_p$ can be solved as:

$$C_p = \frac{w_{pi}C_{pi}}{w_b + w_{si}C_{si}N_sK_s + S_bN_bK_b} \tag{16}$$

From Eqs. (5), (7), (8), (10) and (16), the following relationship concerning $X$ is derived as:

$$X^2 - \frac{w_b/K + N_mS_b\theta_b + w_{si}C_{si}}{S_b}X + \frac{N_m\theta_b w_{si}C_{si}}{S_b} = 0 \tag{17}$$

By solving Eq. (17) in terms of $X$, $X$ is expressed as:

$$X = \frac{(w_b + KN_mS_b\theta_b + Kw_{si}C_{si}) - \sqrt{(w_b + KN_mS_b\theta_b + Kw_{si}C_{si})^2 - 4K^2N_mS_b\theta_b w_{si}C_{si}}}{2K^2N_mS_b\theta_b w_{si}C_{si}} \tag{18}$$

From Eqs. (5) and (6), the removal efficiency, $\eta$, of blue-green algae is rewritten as:

$$\eta = \frac{S_bX}{w_{si}C_{si}} \tag{19}$$

From Eqs. (18) and (19), the calculated value of $\eta$ can be estimated by the determination of $K$, $K_s$, $N_s$, and $N_m$ by least square method, and the presented model can be verified with the experimental data.

## 3. Experimental

### 3.1. Materials

#### 3.1.1. Blue-green algae

Blue-green algae used in the present study were collected from Lake Ohnuma, Oshima, Hokkaido, Japan. The cells were washed with distilled water and were used for flotation experiments. The cell diameter distribution is shown in Fig. 1 and was measured by laser scattering particle size analyzer (HORIBA LA-300, Japan).

#### 3.1.2. Methylated egg albumin

Preparation method of methylated egg albumin (MeOA) was almost similar to the previous study [18]. Egg ovalbumin (OA) was methylated according to the method reported by Fraenkel-Conrat and Olcott [19]. An aqueous solution of OA (ca. 10 g/L) was prepared, and a 0.1 M HCl solution was added. At pH 4.6 (isoelectronic point of OA), OA was precipitated, and the mixture was centrifuged at 3000 rpm for 20 min. The precipitated OA was washed with methyl alcohol and dispersed in a 100-fold volume of methyl alcohol containing 0.05 M HCl. This solution was stirred for 24 h at room temperature. The methylated OA (MeOA) was collected in a centrifuge at 3000 rpm for 20 min and then washed with distilled water. The MeOA was dialyzed for 24 h to remove methyl alcohol and HCl. The degree of methylation was determined from the change in the number of carboxylic groups before and after methylation by a potentiometric titration [18]. In this study, MeOA with methylation degree of 88% was used.

### 3.2. Experimental setup and procedures

#### 3.2.1. Flotation experimental setup

A schematic diagram of the experimental setup is shown in Fig. 2. The experimental setup used in this study was almost similar to that used in the previous study [20]. A bubble column of 4.4 cm in inside diameter and 0.4 m in height was employed. The column was made of transparent acrylic resin. Sintered glass filter (10–15 μm mean-pore size) was installed as a gas distributor at the bottom of the column. Liquid feed and drain were installed at the center and the bottom of the column, respectively. Pressure taps for measuring gas holdup, $\varepsilon_G$, in the column were installed along the wall at intervals of 25 cm. From the value of the measured $\varepsilon_G$, the surface area production rate, $S_b$, of bubble with in the column was estimated by the following equation [21,22]:

$$S_b = 6A\varepsilon_G(1-\varepsilon_G)^{4.65}\left\{\left(\frac{4}{225}\right)\cdot\frac{(\rho_L-\rho_G)^2g^2}{\mu_L\rho_L}\right\}^{1/3} \tag{20}$$

where $A$, $g$, $\rho_G$, $\rho_L$ and $\mu_L$ are the cross-sectional area of the bubble column, gravitational acceleration, gas density, liquid density and liquid viscosity, respectively.

#### 3.2.2. Flotation experimental procedure

Suspended solution of blue-green algae and MeOA solution were prepared at the desired concentration. The ionic strength of these was controlled at 0.005 M with NaCl. The column was filled with 0.005 M NaCl solution, then, air was supplied from air compressor and was dispersed as bubble through the gas distributor. After

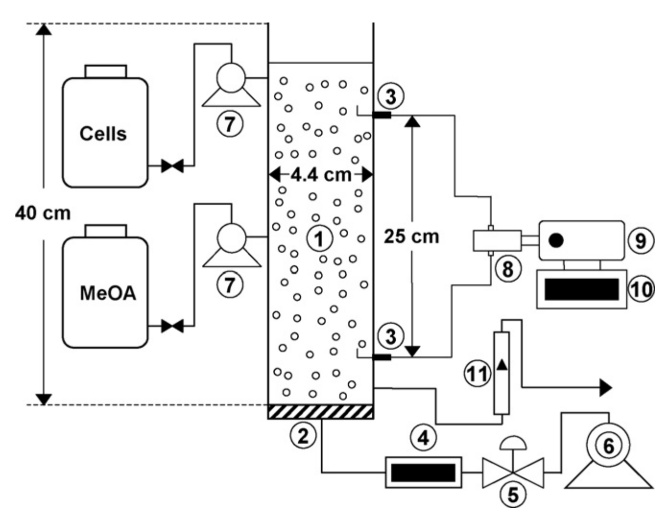


**Fig. 2.** Schematic diagram of experimental setup for blue-green algae removal: (1) bubble column, (2) gas distributor, (3) pressure tap, (4) flow meter, (5) needle valve, (6) air compressor, (7) liquid feed pump, (8) pressure/voltage transducer, (9) amplifier, (10) volt meter, (11) liquid flow meter.

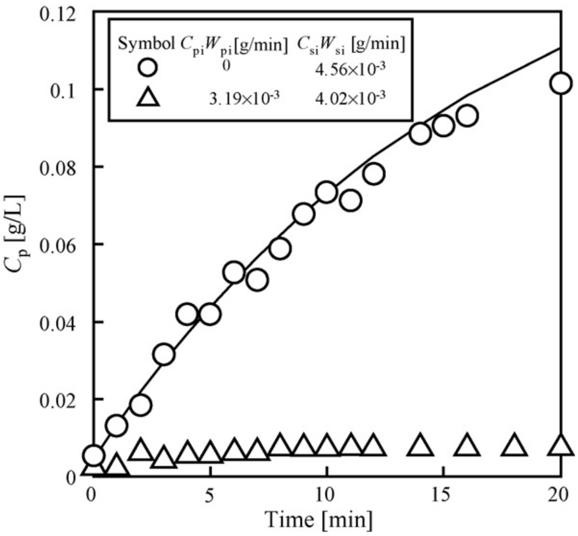


**Fig. 3.** Typical time course of cell concentration profiles within the column with MeOA (triangle) and without MeOA (circle). The superficial gas velocity is $5.37 \times 10^{-2}$ cm/s.

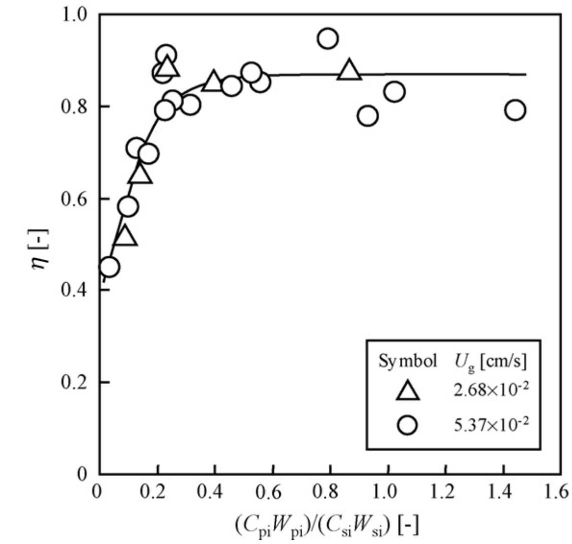


**Fig. 4.** Effect of the ratio of the mass flow rate, $C_{pi}W_{pi}$, of MeOA and the mass flow rate, $C_{si}W_{si}$, of blue-green algae cell on the flotation efficiency, $\eta$.

the confirmation of stable bubbly flow of air within the column, MeOA solution was supplied from the liquid feed at the center of the column by using a quantitative pump. The suspended solution of blue-green algae was fed from the top of the column. The feed volumetric flow rate was 20 cm$^3$/min. To keep the liquid level within the column constant, the volume of the solution within the column as much as MeOA solution and the suspension was drained from the drain tap at the bottom of the column. The drained liquid was sampled at desired time and the concentration of blue-green algae was measured by turbidimetry at 700 nm.

*3.2.3. Determination of adsorption parameters for MeOA*

Two adsorption parameters in Eq. (9), the adsorption equilibrium constant, $K_b$, and the saturated surface density, $N_b$, for MeOA onto bubble surface were determined by continuous foam separation experiment. The experimental setup for foam separation and the procedure was almost similar to our previous study [23].

## 4. Results and discussion

*4.1. Effectiveness of adding MeOA*

Fig. 3 shows a typical cell concentration profile within the column. The open triangle and the open circle correspond to the data obtained with or without feeding MeOA, respectively. The solid line is calculated value from the following equation:

$$C_s = \frac{w_{si}C_{si}}{w_b}\left\{1 - \exp\left(-\frac{w_{si}C_{si}}{V}t\right)\right\} \quad (21)$$

Eq. (21) can be obtained by solving Eq. (4) with the initial condition: $C_s = C_{si}$ at $t = 0$. As seen in Fig. 3, the concentration profile without MeOA (open circle) followed the expected value from Eq. (21). This result indicates that the mixing condition in the present system is regarded as a complete mixing and supports the assumption of neglecting $w_f$ in Eq. (3). On the other hand, the concentration profile with MeOA (open triangle) is rather lower than that without MeOA. This result indicates that blue-green algae cells were removed from the column by adding MeOA.

*4.2. Influence of mass flow rate of cells and MeOA and superficial gas velocity rate on cell removal efficiency*

Fig. 4 shows the removal efficiency, $\eta$, as functions of the ratio of mass flow rate of MeOA and blue-green algae as a typical result. The superficial gas velocity did not affect the removal efficiency significantly. On the other hand, the ratio $(C_{pi}W_{pi})/(C_{si}W_{si})$, of the both mass flow rates affected the removal efficiency up to ca. 0.4. In the range of $(C_{pi}W_{pi})/(C_{si}W_{si}) > 0.4$, the value of $\eta$ was mostly constant, although some scatters are observed. This indicates that the dosage of MeOA should be enough to remove the cells. The influence of $C_{pi}W_{pi}$ on $\eta$ is shown in Fig. 5. As shown in this figure, the $C_{si}W_{si}$ value is constant (ca. $4.0 \times 10^{-3}$ g/min). As seen in Fig. 5, the value of $C_{pi}W_{pi}$ affected strongly on $\eta$. This reason can be explained by the functions of MeOA molecule as frother and flocculant.

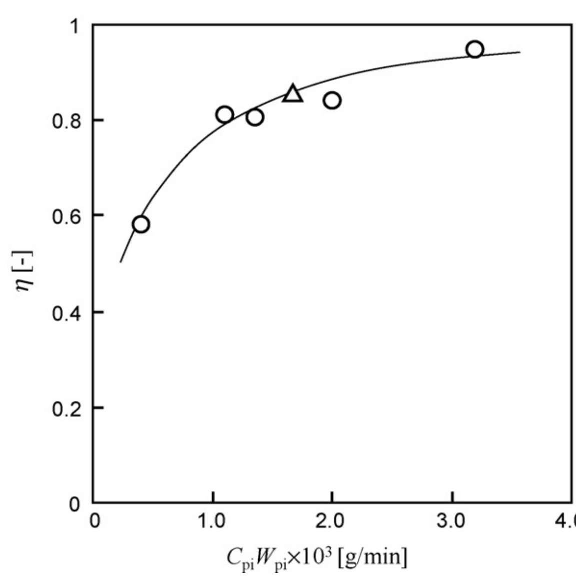


**Fig. 5.** Effect of the mass flow rate, $C_{pi}W_{pi}$, of MeOA on the flotation efficiency, $\eta$. The superficial gas velocity for each symbols are similar to that shown in Fig. 4.

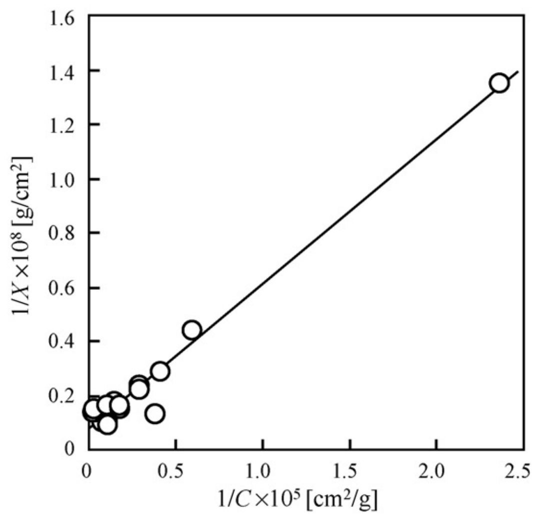


**Fig. 6.** Langmuir plot of MeOA for the determination of the adsorption parameters, $K_b$ and $N_b$.

*4.3. Verification of the proposed model*

The adsorption parameters, $K_b$ and $N_b$, for MeOA adsorbed onto bubble surface were determined by a continuous foam separation method [23]. The adsorption equilibrium relationship could be expressed by Langmuir adsorption isotherm. The Langmuir plot is shown in Fig. 6. $X$ in the coordinate of Fig. 6 corresponds to $W_{f0}(C_{f0}-C_b)/S_b$, in which $W_{f0}$ and $C_{f0}$ represent the volumetric flow rate of the liquid in the foam within the column and the concentration of MeOA in the foam liquid at liquid–foam interface, respectively, and $C_b$ is the equilibrium concentration of MeOA within the column. By measuring the axial distributions of the volumetric flow rate of the liquid in foam and the concentration of MeOA in the foam liquid, $W_{f0}$ and $C_{f0}$ were determined by the extrapolation graphically, respectively [23]. From the values of the intercept and the slope, $K_b$ and $N_b$ were determined as 14.9 dm$^3$/g and $1.26 \times 10^{-2}$ g/m$^2$, respectively.

Fig. 7 shows the comparison of the experimental value of $\eta$ and the calculated value of $\eta$. The estimated adsorption parameters are summarized in Table 1. The data shown in Fig. 7 include the data of Fig. 4, in addition, data for $U_g = 1.42 \times 10^{-2}$ and $1.10 \times 10^{-2}$ cm/s are shown in Fig. 7. The experimental conditions for all data in Fig. 7 of $C_{pi}W_{pi}$ and $C_{si}W_{si}$ were varied from $1.8 \times 10^{-4}$ to $6.42 \times 10^{-3}$ g/min, and, from $6.31 \times 10^{-4}$ to $1.22 \times 10^{-2}$ g/min, respectively. They both were within error 19%. These parameters were estimated by a least square regression except $K_b$ and $N_b$. As seen in Fig. 7, the proposed flotation mechanism in this study was valid fundamentally for the removal process of blue-green algae by using MeOA as frother and flocculant.

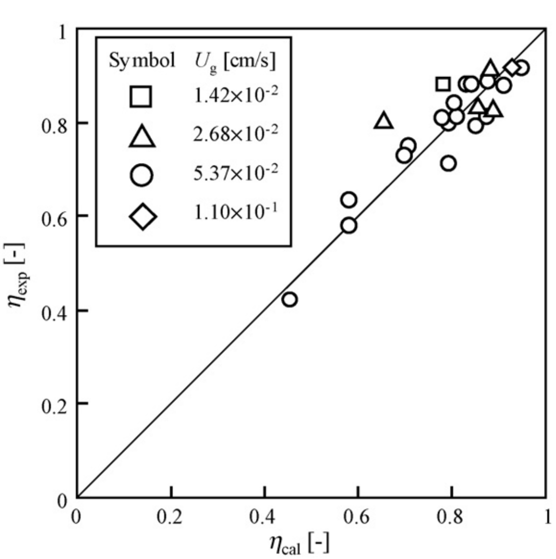


**Fig. 7.** Comparison of the values of the experimental $\eta$ and that of the calculated $\eta$.

**Table 1**
Adsorption parameters for MeOA and blue-green algae.

| $K$ [dm$^3$/g] | $N_m$ [g/m$^2$] | $K_s$ [dm$^3$/g] | $N_s$ [g/m$^2$] | $K_b$ [dm$^3$/g] | $N_b$ [g/m$^2$] |
|---|---|---|---|---|---|
| 1.15 | 1.63 | 1.08 | 1.08 | 14.9 | $1.26 \times 10^{-2}$ |

## 5. Conclusions

The flotation experiments for the removal of blue-green algae were conducted with the methylated egg ovalbumin (MeOA). The flotation model was proposed, which was considered for the adsorption equilibrium relations of MeOA–bubble surface, MeOA–cells surface and cells–bubble surface. From the experimental results, the mass flow rate of MeOA mainly affected the removal efficiency. The good agreement between the experimental $\eta$ and the calculated one can be recognized. The error between the experimental $\eta$ and the calculated one were within 19%.

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