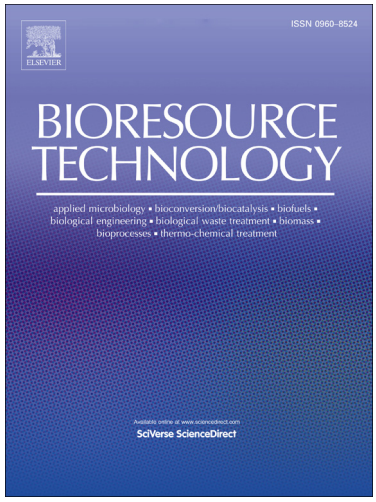

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Dosage effect of cationic polymers on the flocculation efficiency of the marine microalga *Neochloris oleoabundans*

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Key-words: marine microalgae, harvesting, flocculation, mechanism, cationic polymers, dosage effect

Highlights

- Biomass recoveries up to 99 ± 0.8 % were achieved with 20 ppm of flocculant
- A model is developed that describes the recovery as a function of the dosage
- The validated model predicts optimal dosage of flocculant
- Flocculants account for a cost range between 0.15 $/kg$_{biomass}$ and 0.49 $/kg$_{biomass}$

Abstract

A mechanistic mathematical model was developed to predict the performance of cationic polymers for flocculating salt water cultivated microalgae. The model was validated on experiments carried out with *Neochloris oleoabundans* and three different commercial flocculants (Zetag 7557®, Synthofloc 5080H® and SNF H536®). For a wide range of biomass concentrations (0.49-1.37 g L^{-1}) and flocculant dosages (0-150 mg L^{-1}) the model simulations predicted well the optimal flocculant-to-biomass ratio between 43 to 109 $mg_{flocculant}/g_{biomass}$. At optimum conditions biomass recoveries varied between 88% and 99%. The cost of the usage of commercial available flocculants is estimated to range between 0.15 $\$/kg_{biomass}$ and 0.49 $\$/kg_{biomass}$.

1. Introduction

In microalgae cultivation and processing, harvesting using conventional centrifugation and filtration is energy demanding and expensive (Schenk, et al. 2008, Salim et al. 2011, Salim et al. 2012, Milledge and Heaven 2013). Centrifugation of a 0.5 g_{DW}/L suspension using a conventional disk-stack centrifuge for example, requires up to 13.8 MJ/kg_{DW} (Salim et al. 2012). Induced flocculation has been proposed as an effective way for reducing the energy cost considerably (Uduman et al. 2010, Vandamme et al. 2013). By using flocculation as treatment prior to further centrifugation, a 10-fold energy reduction for harvesting the microalgae can be obtained (Salim et al. 2012).

In previous studies, already a variety of flocculants has been tested on microalgae (Vandamme et al. 2013). Flocculation of algae from a marine medium, however, is challenging as ions present in the culture medium shield the flocculant from interaction with microalgae and hinder floc formation (Pushparaj et al. 1993, Uduman et al. 2010, Vandamme et al. 2013). Recently, 't Lam et al. (2014) described the use of cationic polymers for flocculation of marine microalgae. It is described in literature that a microalgal suspension of single cells is stable due to the repulsive forces induced by the charges present on the cell wall (Vandamme et al. 2013). We suggested that the success of cationic polymeric flocculants can be attributed to the ability of these flocculants to interact with individual cells and induce floc formation. Flocs are formed because the cationic groups of the polymeric flocculant adsorb to the negative charged wall of stable cells. The final effect is the destabilisation of the cell suspension (Zahrim 2010, Uduman et al. 2010, Granados et al. 2012, Vandamme et al. 2013). Consequently, both the flocculant and biomass concentrations must affect the performance of flocculation.

The goal of the present study is to characterise and predict this effect of the flocculant dosage on the final biomass recovery obtained after flocculation. Based on experimentally obtained results, a mathematical model is developed to predict the optimal flocculant dosage required at different biomass concentrations. To test and validate the model three different commercially available cationic polymeric flocculants were used to flocculate *N. oleoabundans* cultivated under marine conditions.

2. Materials and methods

2.1 Microalgal strain and cultivation

Neochloris oleoabundans UTEX1185 was cultivated in salt water medium. The composition of the medium was: NaCl: 448.3 mM; KNO_3: 16.8 mM; Na_2SO_4: 3.5 mM; HEPES: 100.1 mM; $MgSO_4.7H_2O$: 5.0 mM; $CaCl_2.2H_2O$: 2.4 mM; K_2HPO_4: 2.5 mM; $Na_2EDTA.2H_2O$: 80 µM; $MnCl_2.4H_2O$: 20. µM; $ZnSO_4.7H_2O$: 4.0 µM; $CoCl_2.6H_2O$: 1.0 µM; $CuSO_4.5H_2O$: 1.0 µM; $Na_2MoO_4.2H_2O$: 0.1 µM; NaFeEDTA: 28 µM. Constant supply of fresh biomass was ensured by cultivation of the microalgae in an Applikon 2L fermentor (Applikon, the Netherlands), operated at chemostat conditions. Continuous stirring at 175 rpm and air sparging at a flow of 7 L/min was applied. The temperature was controlled at 25 ± 0.1 ˚C and the pH was kept at 7.5 by CO_2 supply. The reactor was continuously illuminated with LED lamps at 625 nm with an average incident light intensity of 244 $\mu mol.m^{-2}.s^{-1}$. The microalgae were collected in a dark harvesting vessel and stored at 4 ^oC for one day before the flocculation experiments were performed.

The biomass concentration in the reactor was monitored via daily analysis of the optical density at 750 nm. At various moments, samples were taken. The biomass

dry weight of these samples was determined according to Lamers et al. (2010). Using these biomass concentrations, an OD_{750} versus DW curve was made for determination of the biomass concentrations based on the OD_{750}.

2.2 Flocculants

The polymeric flocculants Zetag 7557® (provided BASF, Germany), Synthofloc 5080H® (provided by Sachtleben, Germany) and SNF H536® (SNF-Floerger, France) were used. These flocculants are often used in the wastewater industry (Renault et al. 2009). All the flocculants are commercial available polyacrylamide-based flocculants with quoted high cationic charge density and polymer length. Stock solutions (1000 ppm) of each flocculant were made in de-ionized (Milli-Q®) water and stored in the dark at 4 ˚C.

2.3 Flocculation tests

In this study a standard flocculant mixing protocol was used (Bilanovic et al. 1988, Divakaran and Pillai 2002, Vandamme et al. 2010, Granados et al. 2012). 10 ml homogeneous samples were taken in duplicate at an optical density OD_{750} of: 0.70 ± 0.1. This OD_{750} corresponds with a biomass concentration of DW of 0.46 ± 0.06 g/L. The samples were transferred into a beaker glass and stirred using a magnetic stirrer at a stirring speed of 500 rpm. Flocculant was added from the stock solutions to the stirred suspension using pipetting at a dosage that varied between 0 and 100 ppm. After addition of the flocculant, the mixture of biomass and flocculant was stirred for 5 minutes at a stirring speed of 500 rpm and subsequently gently mixed at 100 rpm for 10 minutes.

After mixing, 4 ml samples were transferred into 4 ml polystyrene cuvettes (10x10x45 mm, Sarstedt AG&Co). During the 2 hours sedimentation time the OD_{750} was

measured in the upper layer of the cuvette at 20 seconds intervals. The recovery was calculated according to (Salim, et al. 2011):

$$Recovery\ (\%) = \frac{OD_{750}(t_0) - OD_{750}(\mathrm{t})}{OD_{750}(t_0)} * \ 100$$

2.4 Modelling and parameter determination

The computational scripts for the mathematical model were made in Mathworks Matlab 2013a. The model has three variable input parameters: biomass concentration, flocculant dosages and cell diameter. The variable input parameters were experimentally determined. To convert the optical density OD_{750} to the particles concentration (number of particles/µL), a conversion factor is needed. This conversion factor was determined using cell counting with a Coulter counter (Multisizer 3, Beckman). All the experiments were performed in both technical and biological duplicates.

To determine the diameter of the *N. oleoabundans* cells, the Coulter counter (Multisizer 3, Beckman) was used according to the method described by de Winter et al. (2013).

Next to the input parameters, the model also has four different collision rate constants. These constants were fitted using a sum of squared errors method with the experimentally results obtained with the cationic polymers Zetag 755 and, SNF H536 at a flocculant dosage ranging between 0 to 100 ppm and a fixed initial biomass concentration of 0.46 ± 0.06 g/L.

2.5 Model validation

After determining the kinetic parameters by fitting the model on the experimental data obtained with two flocculants (Zetag 7557 and SNF H536), model simulations were first compared with the experimental data obtained with a third flocculant (Synthofloc 5080H) at the same biomass concentrations and flocculant dosages (0.46 ± 0.06 g/L and flocculant dosage ranging between 0 and 100 ppm).

After this initial validation, flocculation experiments were performed at higher biomass concentrations for all three different flocculants. The used flocculant dosages were 50, 100 and 150 ppm and the used biomass concentrations were 0.46; 0.91 and 1.37 g/L (OD_{750} of 0.8, 1.6 and 2.4) resulting in 9 experimental points per flocculant. The 27 experimental points were compared with the predicted biomass recoveries using the model. The relative error between the experimental data and the predicted biomass recoveries were calculated:

$$\partial_x(\%) = \frac{R_{experimental} - R_{predicted}}{R_{experimental}} * 100$$

3. Results and Discussion

3.1 Effect of the flocculant dosage on the biomass recovery

Based on the results of the screening of polymeric flocculants two cationic polymeric flocculants were selected for further study on predicting the effect of flocculant dosage on final biomass recovery obtained after flocculation (‘t Lam, et al. 2014). The biomass recovery after 2 hours of sedimentation was determined as a function of the flocculant dosage, (Figure 1). Both flocculants showed a similar trend; a fast increase of biomass recovery is observed upon increasing the flocculant dosage from 0 to 20 ppm. The biomass recovery is about constant at 20 to 50 ppm, followed by a decreased recovery at dosages higher than 50 ppm.

At a biomass concentration of 0.46 g/L, dosages of 20 to 50 ppm represent a dosage range of 43 ± 0.6 $mg_{flocculant}/g_{biomass}$ and 109 ± 1.4 $mg_{flocculant}/g_{biomass}$. The recoveries and dosages in this study are similar to the recoveries obtained by others under freshwater conditions. Vandamme et al. (2010) used cationic starch as a polymeric flocculant to harvest the freshwater species *Parachlorella kessleri.* After 30 minutes of sedimentation the reported recoveries are higher than 80% using 167 to 200 $mg_{flocculant}/g_{biomass}$. Similar recoveries were reported by Banerjee, et al. (2013). In their study synthesized cationic guar gum was used to flocculate the freshwater algae *Chlorella sp.* at an initial biomass concentration of 0.78 g/L and *Chlamydomonas sp.* at an initial biomass concentration of 0.89 g/L. With a dosage of 51 $mg_{flocculant}/g_{biomass}$ and 112 $mg_{flocculant}/g_{biomass}$, recoveries of 94.5% and 92.15% were obtained after 30 and 15 minutes of sedimentation.

Similar results at seawater salinities where obtained in the study of Farid et al. (2013). By using cationic polymeric flocculants, the marine microalgae *Nannochloropsis sp.* was harvested with biomass recoveries of 80%. As a flocculant, they used modified chitosan in combination with a pH-increase. Although this approach was successful, it is not known if the induced flocculation is caused merely by the modified chitosan or by the pH-increase. At elevated pH, divalent salts such as calcium and magnesium salts can precipitate and 'sweep' the algal biomass which causes an enhanced biomass recovery (Vandamme et al. 2013).

To understand the decrease in biomass recovery obtained at elevated flocculant dosages that is presented in Figure 1, we propose the mechanism presented in Figure 2.

Figure 2 depicts the mechanism describing the starting of the floc formation: a small amount of flocculant reversibly adsorbs on a part of the cell wall, resulting in a locally destabilised cell. These destabilised cells can collide with each other or with other cells. This results in the formation of the first floc consisting of a few cells (Gregory 1973, Mabire et al. 1984, Rattanakawin and Hogg 2001). The floc can further grow under the same sequence of events of destabilisation and collision.

Exceeding amount of flocculant, however, can cover the whole cell wall, resulting in a reversion of the charge of the particle. This results in a stabilized cell suspension due to the repulsive forces among the adsorbed polymers. Eventually, this class of particles are inhibited to further flocculate.

Although this phenomenon has already been proposed in previous experimental studies (Gregory 1973, Mabire et al. 1984, Tenney et al., 1969), existing flocculation

models (Runkana et al. 2004, Thomas et al. 1999, Rattanakawin and Hogg 2001) do not include the formation of these inhibited particles. Our model takes over-coverage of the cell wall by the flocculant into account.

The sequence of destabilisation and collision is a chain of events in which a recent formed floc will be destabilized again in order to further collide forming a new, larger floc. Eventually, this chain of events will result in a steady state. At this steady state, a constant particle size distribution will be present.

3.2 Model development

The proposed mechanism of Figure 2 was incorporated in a mathematical model. This model was used to understand the effect of the flocculant dosage. In the model, three different classes of particles are taken into account: stable particle (C^S), destabilised particle (C^D) and inhibited particles (C^I). The particle size distributions of these classes are described classifying the particles by their number of cells. The detailed translation of the mechanism into a model is presented in the Appendix.

Both individual microalgal cells and formed flocs are considered as rigid homogeneous spherical particles. The density of all the particles present is equal and the size of the flocculant molecules is not taken into account to calculate the size/mass of a floc.

For each population of particles, the reactions involved in the flocculation mechanism i.e.: **a**dsorption, **d**esorption, **i**nhibition and **f**locculation, are assumed to follow first order kinetics with kinetic constants, respectively: β^A, β^D,β^I, β^F.

We hypothesized that the values of β depend on the sizes of particles involved in the specific step of the flocculation network.

The adsorption constant β_i^A is dependent on the surface area of the particles. With an increasing particle size, the chance of a flocculant being adsorbed on the surface is increasing as well.

$$\beta_i^A = \beta_0^A \cdot {d_i}^2$$

Desorption is often dependent on the absorbed quantity. However, in our model the desorption rate is limited by the mass transfer of flocculants through the external layer surrounding the particle. Therefore, β_i^D is assumed to be independently of the particle size, and constant:

$$\beta_i^D = \beta_0^D$$

The formation of inhibited particles depends by the capacity of the flocculant to completely cover the destabilised particle “i”. With an increasing particle surface, more flocculant are needed. The chance of forming inhibited particles thus decreases with increasing particle surface areas and the rate constant β_i^I is described by

$$\beta_i^I = \beta_0^I \cdot \frac{1}{{d_i}^2}$$

In which β_0^I is the maximum inhibition constant.

The flocculation of the particles is assumed to follow the orthokinetic collision mechanism as described by Smulochowski (1917). With orthokinetic collisions, the volume of the particles is the predominant factor influencing the collision rate. The rate constant for formation of larger flocs β_{ij}^F is thus expressed in terms of the volume of the individual particles involved.

$$\beta_{ij}^{F} = \frac{G}{6}\,(d_i + d_j)^3$$

From the reaction network and the involved kinetics, the mass balances are derived for each element 'i' of the three classes. According to the mass balances, the elements element 'i' and 'j' can collide forming larger particles of class 'k' up to the largest size class 'N'.

$$\frac{dC_i^S}{dt} = \beta_i^D * C_i^D - \beta_i^A * C_i^S * C_F - \left(\sum_{j=1}^{j=N} \beta_{ij}^F * C_j^D\right) \cdot C_i^S + \sum_{k=1}^{k=i-1} \beta_{k(k-i)}^F * (C_{k-1}^S + C_{k-i}^D) * C_k^D$$

$$\frac{dC_i^D}{dt} = \beta_i^A * C_i^S * C_F - \beta_i^D * C_i^D - \beta_i^I * C_i^D * C_F - (\sum_{j=1}^{j=N} \beta_{ij}^F * (C_j^S + C_j^D)) * C_i^D$$

$$\frac{dC_i^I}{dt} = \beta_i^I * C_i^D * C_F$$

Finally the mass balance of the flocculant is also taken into account:

$$\frac{dC_F}{dt} = \sum_{i=1}^{i=N} \beta_i^D * C_i^D - \beta_i^A * C_i^S * C_F - \sum_{i=1}^{i=N} \beta_i^I * C_i^D * C_F$$

To determine the biomass recovery from the population balances, it is assumed that after flocculation all the particles present at least in the second size class of the particle size distribution are able to settle (Tenney et al. 1969).

The experimental results obtained with Zetag 7557 and SNF H536 (Figure 1) are used to fit the four collision rate constants. Next to the fitting, the input parameters (OD_{750}-cell number conversion factor, C_i, C_F and d_{cell}) of the model were experimentally determined (Table 1). An overview of the input parameters and the determined values of the collision rate constants are provided in Table 1.

With the fitted values for the kinetic constants the model was used to predict biomass recoveries at different flocculant dosages. After fitting the average relative errors between predicted and observed biomass recovery was 5% for SNF H536 and 8% for Zetag 7557.

3.3 Model validation at one biomass concentration

The goal of the model is to describe a general trend in the biomass recovery as a function of the flocculant dosage that is applicable for a large variety of cationic polymeric flocculants. Therefore, after the calibration of the model with the flocculants Zetag 7557 and SNF H536, the model was used to simulate the biomass recoveries at different dosages of a third flocculant at a constant biomass concentration of 0.46 g/L (Figure 3). As a third flocculant Synthofloc 5080H was used. Figure 3 shows that a decrease in biomass recovery at elevated dosages is observed, just as with the other flocculants (Figure 1). The model showed a similar trend in predicting the biomass recoveries as a function of the flocculant dosage.

The predicted biomass recoveries are on average slightly higher than the experimental results with a relative error of 15%. This error is caused by our assumption that all flocculants behave similar. This resulted in similar parameters for the use of different flocculants. In reality these input parameters may deviate causing differences in predicted biomass recoveries. However, at the optimal flocculant dosage range of 20 to 50 ppm, the relative error is only 5%. This illustrates the ability to predict optimal flocculant dosages for multiple flocculants with the model.

3.4 Model validation at different biomass concentrations

Although the model is in agreement with the experimental data obtained at an initial DW of 0.46 ± 0.06 g/L, it is not known if the input parameters are also valid when higher biomass concentrations are applied. Additional simulations were therefore performed at an initial DW of 0.46 ± 0.06 g/L, 0.91 ± 0.005 g/L and 1.37± 0.005 g/L and at 50 100 and 150 ppm and compared with experimental data obtained with all three flocculants (Figure 4).

In Figure 4, the flocculant dosages and used biomass recoveries of the 27 experimental points were used to calculate a flocculant-to-biomass ratio ($mg_{flocculant}/g_{biomass}$). By doing so, we were able to present all the experimental data, obtained at different biomass concentrations in a single figure.

According to the proposed mechanism there is an optimal ratio between flocculant dosage and biomass recovery. When this ratio is exceeded, the recoveries are decreasing again. This is in accordance with both the experimental and simulated optimal dosage of 70 $mg_{flocculant}/g_{biomass}$, followed by a decrease in biomass recovery.

The biomass recoveries obtained with Zetag 7557 are lower than the predictions at dosages from 70 $mg_{flocculant}/g_{biomass}$ onwards. In addition, these recoveries are also lower than the biomass recoveries obtained with the two other flocculants. This illustrates, that although only cationic polyacrylamides were used as flocculants, the individual flocculants have different flocculation properties. In this model, no flocculant characteristics were included. All flocculants were considered as equal.

To simulate properties of different flocculants in harvesting marine microalgae, the model should be extended by including some flocculant unique parameters. This can

be done by including these characteristics in the kinetic constants ('β'). Implementing flocculant dependent characteristics can be done by replacing the collision rate parameters '$\beta_0^D, \beta_0^A,\ \beta_0^I$ and G' for other expressions. A possible improvement in the collision rate parameters is including flocculant dependent parameters such as flocculant length or cationic charge density. When these specific parameters are included, no generalisation in cationic polymers is needed, which might result in a more accurate prediction of biomass recoveries.

The goal of the model was to confirm our proposed mechanism in order to create a better understanding of marine flocculation. In addition, the model can be used to perform some basic simulations to estimate the optimal flocculant dosage. Based on the average relative error of 15% that is calculated by including all 27 experimental points, it appears that the proposed mechanism is in agreement with the experimental observations. This novel model can be used to simulate flocculation efficiency at flocculant limiting conditions (low dosages). Moreover, it also predicts the flocculant inhibiting effects at dosages that exceed the optimal flocculant dosage ratio. This model is there for usable in further evaluations to determine optimal flocculant dosages

3.5 Evaluating marine flocculation

Both the experimental work and simulations presented in this study shows that the optimum flocculant dosage to harvest the microalgae *N. oleoabundans* cultivated in saltwater medium ranges between 43 to 109 $mg_{flocculant}/g_{biomass}$. In other applications of cationic polymers such as removal of contaminants in the wastewater industry, the flocculant demand is 5 to 10 times lower (Lee et al., 2010, Wong et al. 2006).

Although it is possible to harvest marine microalgae using flocculation, it is not known yet if flocculation can contribute in lowering the cost price of algal harvesting. Wong et al. (2006) used in his study multiple commercial available cationic polyacrylamides. According to his study, the cost price of cationic polyacrylamides ranges between 3.45 and 4.50 $/kg. When the optimal flocculant dosage of 43 to 109 $mg_{flocculant}/g_{biomass}$ is taken into account, the price of 1 kg of harvested biomass would range between 0.15 $/$kg_{biomass}$ and 0.49 $/$kg_{biomass}$. In this estimation only the flocculant usage is taken into account.

This cost estimation is slightly higher than the evaluation reported in the screening study of Roselet et al. (2015). In that screening, using the marine microalgae *Nannochloropsis oculata* and cationic polyacrylamides, the applied flocculant dosage was slightly lower than the dosages applied in this study: between 18 and 27 $mg_{flocculant}/g_{biomas}$. This dosage resulted in a chemical cost up to 0.22 $/$kg_{biomass}$ for cationic poly(acry)amides (Roselet et al., 2015). These results underline that the flocculant demand can account for a considerable cost in harvesting.

To decrease the flocculant demand and belonging costs of the flocculants, more efforts should be taken to further understand the role of the flocculant in inducing flocculation. When this role is known, optimized flocculants can be designed that are

suited to the requirements of microalgal flocculation. Optimized flocculants will result in a lower necessity of flocculants to destabilise cells, while maintaining the biomass concentration (Roselet et al., 2015). One example of flocculant optimization is the study of Morrissey et al. (2014). In that study, recyclable flocculants have been proposed. Although this is not a direct optimization of the flocculant resulting in a lower optimal dosage ratio, the recyclability of the flocculant will result in a lower flocculant demand. Next to the development of recyclable flocculants, new types of flocculants such as cellulose nanocrystals have been reported. These type of flocculants are potentially cheaper than current studied poly(acryl)amidic ones, and may be feasible for in food- and feed applications (Eyley et al., 2015).

4. Conclusions

In this study an experimental and modelling approach were used to propose a mechanism for flocculation. The mechanism enabled us to understand flocculation under various conditions. By predicting optimal flocculant dosages and comparing with the experimental results, this study revealed that there is an optimal ratio between flocculants and biomass that determines the needed amount of flocculant at various biomass concentrations. For *N. oleoabundans* this ratio is between 43 to 109 $mg_{flocculant}/g_{biomass}$. Although this is similar to dosages reported in other micro algal studies, it is approximately 10 times higher than the dosage used in the wastewater industry.

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Figure 1: Biomass recoveries after 2 hours of sedimentation with Zetag 7557(■) and SNF H536 (▲). Error bars represent biological replicates (n=2).

Figure 2: Schematic overview of the interactions between algal cell particles and the flocculant.

Table 1: Input of the model: Next to the four collision rate constants ('β') that were determined using experimental determined data, also the biomass specific parameters (cell size and cell number conversion factor) are presented.

Figure 3: Experimental data with initial DW of 0.46 ± 0.06 g/L (●). All the experimental results were obtained in biological replicates (n=2) by using the flocculant Synthofloc 5080H. The simulations are performed with the fitted collision rate constants and similar biomass concentration.

Figure 4: Comparison between simulations and experimental according to the study design. Applied biomass concentration 0.46 g/L, 0.91 g/L and 1.37 g/L. Solid line are predicted biomass recoveries and Zetag 7557(♦), Synthofloc 5080H (■) and SNF H536 (▲) . Error bars represent biological replicates (n=2).

Parameter	value	Unit
OD_{750}-Cell number conversion factor	23192	$OD_{750-\frac{cells}{\mu L}}$
C_i	variable	$\frac{g}{L}$
C_F	variable	ppm
d_{cell}	3.5	μm
β_0^D	$0.631 * 10^{-5}$	$\frac{1}{second}$
β_0^A	$1.035 * 10^{-5}$	$\left(\frac{1}{second}\right) * \left(\frac{L}{\mu gram}\right) * \left(\frac{1}{\mu m^2}\right)$
β_0^I	$0.589 * 10^{-5}$	$\left(\frac{1}{second}\right) * \left(\frac{L}{\mu gram}\right) * \mu m^2$
G	$0.008 * 10^{-5}$	$\left(\frac{1}{second}\right) * \left(\frac{\mu L}{number\ of\ particles}\right) * \left(\frac{1}{\mu m^3}\right)$