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

G.P. 't Lam [a,*], E.K. Zegeye [a], M.H. Vermuë [a], D.M.M. Kleinegris [b], M.H.M. Eppink [a], R.H. Wijffels [a,c], G. Olivieri [a]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[b] Wageningen UR, Food & Biobased Research, AlgaePARC, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[c] University of Nordland, Faculty of Biosciences and Aquaculture, N-8049 Bodø, Norway

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}$.

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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 and 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, 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 et al., 2010;

* Corresponding author.
E-mail address: gerard.tlam@wur.nl (G.P. 't Lam).

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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 *Neochloris oleoabundans* cultivated under marine conditions.

2. Methods

2.1. Microalgal strain and cultivation

N. 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; $MgSO_4 \cdot 7H_2O$: 5.0 mM; $CaCl_2 \cdot 2H_2O$: 2.4 mM; K_2HPO_4: 2.5 mM; $Na_2EDTA \cdot 2H_2O$: 80 μM; $MnCl_2 \cdot 4H_2O$: 20 μM; $ZnSO_4 \cdot 7H_2O$: 4.0 μM; $CoCl_2 \cdot 6H_2O$: 1.0 μM; $CuSO_4 \cdot 5H_2O$: 1.0 μM; $Na_2MoO_4 \cdot 2H_2O$: 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 μmol m^{-2} s^{-1}. The microalgae were collected in a dark harvesting vessel and stored at 4 °C 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 and Shelef, 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: 0.7 ± 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 min at a stirring speed of 500 rpm and subsequently gently mixed at 100 rpm for 10 min.

After mixing, 4 ml samples were transferred into 4 ml polystyrene cuvettes (10 × 10 × 45 mm, Sarstedt AG&Co). During the 2 h sedimentation time the OD_{750} was measured in the upper layer of the cuvette at 20 s intervals. The recovery was calculated according to (Salim et al., 2011):

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

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 and 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:

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

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 h of sedimentation was determined as a function of the flocculant dosage (Fig. 1). Both flocculants showed a similar trend; a fast increase of biomass recovery is observed