https://doi.org/10.18321/ectj728

Concentration of *Chlorella Sorokiniana* Microalga Biomass at Combined Usage of Coagulants and Flocculants

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Article info

Received:
14 January 2018

Received in revised form:
27 March 2018

Accepted:
16 June 2018

Keywords:
coagulation
flocculation
chitosan
polyacrilamide
iron (III) chloride
microalga *Chlorella Sorokiniana*
precipitated biomass

Abstract

In this work there were studied various methods for concentrating the algae biomass, and mechanisms of concentration processes of the microalgae cells. Presented are papers devoted to usage of various coagulants and flocculants (iron (III) chlorides and sulphates, titanium tetrachloride, slaked lime, potassium chloride, potassium permanganat, aluminum potassium sulphates, Flopam FO 4550 SH). In the experimental part there is studied the usage of $FeCl_3*6H_2O$ coagulant in the amount of 4, 6, 8, 10, 20, 30 mg/l of microalga *Chlorella Sorokiniana* suspension. Microstructure investigations have shown that at addition of ferrous chloride of concentration more than 20 mg/l, the cell death is more than 50%. Concentrations from 4 to 10 mg/l achieve low degree of precipitation (not more than 18%). Aiming at increasing degree of precipitation we investigated usage of two-component system coagulant-flocculant. Usage of two-component system results in a significant increase of precipitation degree of microalga *Chlorella Sorokiniana*: at addition of chitosan the degree reaches 53%, at addition of PAA it is 79%. It was determined the volume of the thickened biomass sludge. It shows that usage of chitosan results in more tightened biomass layer, which occupies minimal volume (V = 140 ml). The following three-component mixture is an optimum variant for high-efficient precipitation of biomass: coagulant $FeCl_3$ in the amount of 6 mg/l; flocculants as a mixture of chitosan 2% and PAA 1% in the amount of 10 ml/l. Addition of chitosan solution as a flocculant results in decrease of pH value, which is caused by usage of acetic acid for preparation of chitosan solution. Microstucture analysis of the precipitated biomass shows that at coloration of *Chlorella Sorokiniana* cells by methylene blue, the amount of dead (colored) cells is not less than 5%. Consequently, the precipitated biomass might be used for obtaining of valuable components.

1. Introduction

A problem of biomass concentrating for its further implementation arises at industrial cultivation of microalgae. Suspension of microalgae slowly precipitates in a nutrient broth when it is cultivated at photobioreactors.

Microalgae have negative charge of cell surface and small cell dimensions, so its natural precipitation rates are rather slow. For cell precipitation various methods are used, including coagulation and flocculation. The process of aggregation of separate cells into larger flakes is based on the process of neutralization of similar charges at the particle surfaces. In case of algae it takes place at the cell surface. Coagulation process might be initiated by addition of salts of coagulatory metals (Al^{3+}, Fe^{3+}), which hydrolize in liquid and neutralize surface charge of algae. At high pH value the metal hydroxides are formed, which are prone to precipitate at the flakes and make physical connections between algae, and this leads to increased biomass density [1, 2].

In the function of flocculants one may use special polymers, which are adsorbed on the cell envelope surface. These polymers stabilize electronegative cell charge and form connections between cells [3, 4].

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A vast number of papers are devoted to investigations of coagulation and flocculation processes. The authors [1] studied influence of 8 various flocculants on concentrating efficiency for marine microalga Nannochloropsis oculata. Iron (III) chlorides and sulphates have shown the highest degree of microalgae precipitation. After 180 min from addition of these salts in concentrations of 0.4 and 0.6 g/l the precipitation degrees were 93.80% and 87.33%, respectively. At the given concentration iron salts induced total cell lysis. Zinc salts showed high flocculation efficiency: $ZnCl_2$ (0.6 g/l) – 89.12% and $ZnSO_4$ (0.8 g/l) – 84.17% at 210 and 240 min, respectively. Aluminum salts showed the following flocculation efficiency: 85.46% for $AlCl_3$ (0.6 g/l) and 82.27% for $Al_2(SO_4)_3$ (0.4 g/l) at 210 and 240 min, respectively.

In the works of H. Nakamura [2] as a chemical coagulant the authors used titanium tetrachloride in concentration of 0.001%, as well as 10%-solution of manganic sulphate. They showed that slaked lime (1%-solution) after 4–6 h from addition into suspension in the ratio 50–70 ml/l caused complete alga cell precipitation onto basin bottom. However, according to N.P. Aruytunyan [5], calcium negatively affects the cell envelope and changes its permeability. Tambov scientists [6] used the following reagents for microalgae precipitation: acetic acid, alkali, slaked lime, potassium chloride, potassium permanganat, aluminum potassium sulphates. They concluded that these chemical substances significantly decreased biomass quality and had low efficiency during cell precipitation in suspension. The authors also investigated influence of Flopam FO 4550 SH flocculant on microalga biomass precipitation degree. Microscopic studies have shown that more than 50% of biomass appeared to be not viable. Basing on the aforesaid results the authors suggest to use centrifuging and separation for biomass concentrating in order to achieve the complete cell precipitation.

The aim of this work was to choose optimum quantitative and qualitative composition of reagents (coagulants, flocculants) for high-speed concentrating of microalgae *Chlorella Sorokiniana* biomass.

2. Materials and methods

For our investigations it was used microalgae *Chlorella Sorokiniana*, its cultivation conditions are presented in [7–9]. It studied biomass concentration processes as follows: microalga *Chlorella Sorokiniana* suspension with optical density 1.160 was put in seven cylinders (volume of a cylinder is 500 ml, diameter is 90 mm, height is 390) together with reagents (coagulants, flocculants).

Coagulant:

- Iron chloride $FeCl_3*6H_2O$ in the amounts of 4, 6, 8, 10, 20, 30 mg/l;

Flocculants:

- Polyacrilamide (PAA) solution. For preparation of this solution PAA AK 631 in the amount of 1 g was dissolved in distilled water.
- Chitosan solution. For preparation of this solution 2 g of chitosan was dissolved in 100 ml of acetic acid solution of 1.5% concentration;
- Chitosan and polyacrilamide solution. For preparation of this solution PAA AK 631 in the amount of 1 g was added into the chitosan solution described above.

Schematic course of experiment for usage of coagulants and flocculants for microalga cell concentration is presented in Table 1.

The degree of precipitation and concentration of the microalga cell was estimated by optical density of suspension, taken from supernatant fluid. Precipitation degree was determined from the formula:

$$S = (A_0 - A)/A_0 \cdot 100\%, \quad (1)$$

where A is optical density of the supernatant fluid; A_0 is initial optical density of microalga suspension (A_0 = 1.160).

Optical density was determined using UV 1280 spectrophotometer after 10, 30, 60, 180, 360 min of coagulant and flocculant additions. Microalga suspension pH was determined before and after addition of reagents using ion meter HANNA HI 2211. For calculation the amount of viable microalga cells we carried out microstructure investigations using LOMO microscope after reagent precipitation.

Table 1

Schematic course of experiment for usage of coagulants and flocculants for microalga cell concentration

Cylinder №	$FeCl_3 \cdot 6H_2O$	Chitosan 2% (10 ml/l)	Chitosan 2%+1% PAA (10 ml/l)	PAA 1% (10 ml/l)
0	-	-	-	-
1	0.004 g/l	-	-	-
2	0.006 g/l	-	-	-
3	0.008 g/l	-	-	-
4	0.006 g/l	+	-	-
5	0.006 g/l	-	+	-
6	0.006 g/l	-	-	+

3. Results and discussion

It is known from literature that iron and aluminum compounds are used as coagulants. Nutrient broth for microalga cultivation contains iron (III) chloride, so in our work we used iron (III) chloride as a coagulant. At the first stage we used iron chloride in the concentration of 4, 6, 8, 10, 20, 30 mg/l of *Chlorella Sorokiniana* suspension. Microstructure investigations showed that at addition of iron chloride in concentration more than 20 mg/l, the cell death degree is more than 50%. This fact is proved in literature, where it is shown that usage of high concentrations of iron chloride as a coagulant [1] results in cell lysis and further usage of the precipitated biomass is not rational. Concentrations from 4 to 10 mg/l achieve low precipitation degree (not more than 18%), which doesn't meet the stated requirements. Papers [10–13] show that for suspension precipitation coagulants are more efficient in combination with flocculants. In order to increase precipitation degree one suggest using two-component system coagulant-flocculant. Solutions of chitosan and polyacrilamide possessing high flocculating properties were used as flocculants. For this purpose to the suspension solution we added 6 mg/l of $FeCl_3$ as a coagulant, and flocculants were the following: 1–10 ml/l of chitosan solution; 2–10 ml/l of PAA solution. Usage of two-component system results in an increase of precipitation degree of suspension: at addition of chitosan this degree reaches 53%, at addition of PAA it is 79%. For investigated systems we determined volume of the thickened biomass sludge, which shows that usage of chitosan results in more tightened biomass layer, which occupies minimal volume (V = 140 ml). Biomass tightening is likely to be connected with properties of biopolymer-chitosan, which envelops microalga cells, and makes them more heavy-weight. Floccules gravitate to the cycinder bottom and the sludge gets thicker (Table 2).

Further it was studied the influence of a three-component coagulant system ($FeCl_3$, 6 mg/l) and two flocculants (PAA+chitosan, 8, 10, 12 l per 1 l of microalga suspension). Precipitation degree was determined according to the formula (1). Usage of three-component system results in increase of precipitation degree up to 97% and decrease of biomass volume to 100 ml (Fig. 1).

For samples without chitosan the dense precipitate of the biomass was formed only after 1440 min (24 h), and the boundary wasn't clearly seen. Whereas for samples # 4 and 5 the dense precipitate began to form in 10 min after addition of three-component mixture: chitosan, polyacrilamide, $FeCl_3$. Addition of chitosan solution as a flocculant results in decrease of pH value (Table 2), which is caused by usage of acetic acid for preparation of chitosan solution. Microstructure analysis of the precipitated biomass shows that at coloration of cells by methylene blue, the amount of dead (colored) cells is not less than 5%. Consequently, the precipitated biomass might be used for obtaining of valuable components.

It is seen from Fig. 1 that addition of 10 ml/l of flocculant mixture (chitosan+PAA) results in achieving precipitation degree up to 97% for 10 min. A larger amount of additive (12 ml/l) doesn't significantly increase precipitation degree (up to 98%), but reduces pH values. So we accept 10 ml/l to be optimal value of flocculant additive.

Table 2

Influence of coagulants and flocculants on precipitation degree and biomass sludge volume for *Chlorella Sorokiniana*

Cylinder №	pH after addition of coagulants and flocculants	V of thickened sludge, ml	S, %	Time of complete precipitation, min
0	9.99	400	1.3	1440
1	9.85	380	12	1440
2	9.97	380	18	1440
3	9.86	380	14	1440
4	5.95	140	53	10
5	5.70	100	97	10
6	9.97	360	79	1440

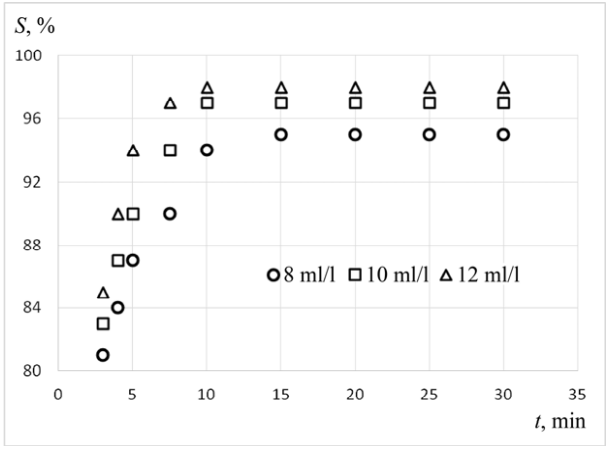
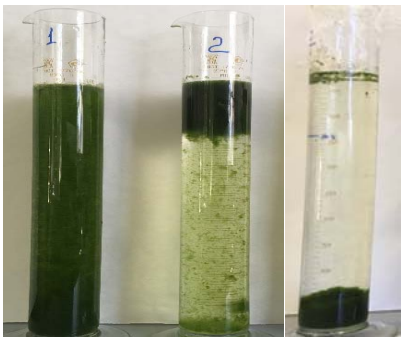


Fig. 1. Relation between precipitation degree (S, %) of *Chlorella Sorokiniana* biomass and time (t) for addition of coagulant (6 mg/l of $FeCl_3$) (everywhere) and flocculant (chitosan+PAA) in the amount of 8, 10, 12 ml/l.

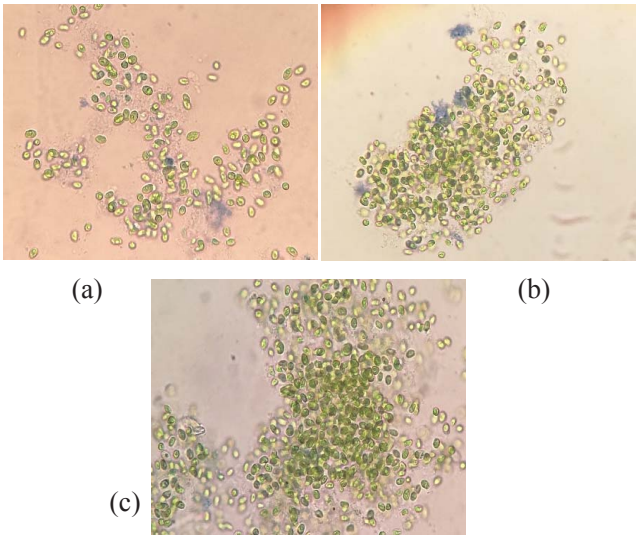
At addition of three-component mixture ($FeCl_3$+chitosan+PAA) during the first 3 min the major biomass part rises to the upper cylinder part (Fig. 2b), as loose flakes with density less than 1.0225 g/cm^3 (nutrient broth density) are formed. After 5 min the biomass densifying takes place up to densities more than 1.0225 g/cm^3 and it gravitates to the cylinder bottom (Fig. 2c).



(a) (b) (c)

Fig. 2. Photographs of *Chlorella Sorokiniana* suspension without (a) and with addition of coagulants-flocculants after 3 min (b) and 10 min (c).

Microstructure analysis has shown that after coloration by methylene blue for 2–3 min the amount of dead cells doesn't exceed 5% (Fig. 3), which states for safety of the additives and possibility to use the concentrated biomass for obtaining valuable components. Biomass microphotographs show agglomerates of higher densities, which were formed after addition of three-component mixture: 6 mg/l of $FeCl_3$ and flocculants (chitosan+PAA) in the amount of 10 ml/l (b) and 12 ml/l (c).



(a) (b)

(c)

Fig. 4. Microstructure study of microalga *Chlorella Sorokiniana* cells after addition of three-component mixture: coagulant (6 mg/l of $FeCl_3$) (everywhere) and flocculants (chitosan+PAA) in the amount of 8 (a), 10 (b), 12 (c) ml/l.

4. Conclusions

The performed work resulted in selection of optimal ratio between coagulant and flocculant amounts for high-efficient concentrating of *Chlorella Sorokiniana* biomass. The following mixture: 6 mg/l of $FeCl_3$ as a coagulant; mixture of chitosan 2% and PAA 1% in the amount of 10 ml/l as flocculants has optimal parameters. Microstructure studies have shown that usage of these reagents is safe for obtaining viable biomass. The amount of dead cells in the concentrated biomass doesn't exceed 5%.

Acknowledgments

This work was performed within the implementation of Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014-2020, the project "Development and implementation of innovative biotechnologies for treatment of microalgae *Chlorella Sorokiniana* and duckweed Lemna minor" (Agreement #14.587.21.0038 of 17.07.2017), the unique project identifier is RFMEFI58717X0038.

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