Flocculation-based methods of microalgae removal from the eutrophic pond enrichment culture

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ABSTRACT

The method of flocculation of biomass from the water depth to the bottom can be applied in case of local algae blooms. In our research, the combined application of FeCl₃ and Polyethyleneoxide-based cationic flocculant was applied for the first time for the harvesting of the enrichment culture obtained from the reservoir during eutrophication. The effects of coagulant (FeCl₃·6H₂O), various flocculants based on polyacrylamide (PAA), polyethyleneoxide (PEO) and flocculated biomass as ballast agent, dosage and sedimental time on flocculation efficiency of harvesting enrichment culture obtained from Pond Chernoiostochinsky during eutrophication have been studied. The results show that the flocculation efficiency achieved about 90% after 5 min of sedimentation when adding coagulant and flocculant mixture (FeCl₃ 30 mg/L + PEO based Sibfloc-718 2.5 mg/L) or flocculant with ballast agent (FeCl₃ 30 mg/L + Sibfloc-718 2.5 mg/L + 1.7% flocculated biomass). PAA, PEO and FeCl₃·6H₂O did not demonstrate a sufficient flocculation capacity with enrichment culture containing different types of microalgae.

Key words: ballast, eutrophication, flocculation, harvesting of biomass, inorganic coagulants, polymeric flocculants

HIGHLIGHTS

• Combined application of FeCl₃ and polymeric flocculant was successfully applied for sedimentation of enrichment culture obtained from an eutrophic pond.
• The use of flocculants has been shown to be a promising method of biomass concentration in water bodies.
• Application of flocculated biomass as ballast agent for further biomass flocculation was shown to be effective.

INTRODUCTION

Anthropogenic effects on aquatic ecosystems around the world are increasing constantly. The eutrophication process starts as a result of excess nutrient inputs to many water bodies (Namsaraev et al. 2018). Gradually, mass development of
photosynthetic organisms (harmful algal blooms, HAB) is becoming one of the biggest problems for fresh water bodies and coastal zones of seas adjacent to areas with large population. The control of mass development, collection and economically efficient utilization of excessive biomass of algae are among the most important directions of control over the eutrophication process and neutralization of its negative effects (Schindler et al. 2016).

Harvesting biomass during algal overgrowth is one of the most technically complicated and expensive operations. Today, the following trends stand out in this area (Pei et al. 2017):

- The use of precipitating reagents;
- The use of precipitating reagents in specialized tanks to concentrate biomass after primary harvest;
- The use of different materials with branched structure or high porosity for mechanical biomass harvesting;
- The use of phosphorus binding reagents;
- Bottom cleaning and/or bottom consolidation.

The application of a particular method depends on the characteristics of the reservoir and the purposes of its use.

Both materials for biomass collection and sorbent materials can be used to control the algal overgrowth of water bodies. Traditionally, mechanical harvesting systems based on the slow movement of membranes or geotextiles through water containing biomass are used. This is especially effective in large open reservoirs. Stationary systems with the natural movement of water pushing it through the filtering material are also used (Pienkos & Darzins 2009). Another method of mechanical harvesting is pumping the water volumes through a water treatment system installed on a watercraft or on land (Hanotu et al. 2012). Filtering through sand filters may be a simple solution (Brink & Marx 2013). However, removing biomass from the filter requires additional washing of the filter bed. In addition, suspended solids, which are then washed with the biomass, remain in the filter. Quite a lot of research has been carried out on the use of different flotation options to collect biomass (Sun et al. 2017).

Metal salts are currently used to bind phosphorus in the reservoir. Among these compounds, aluminium and iron salts have been widely used. The use of modified lanthanum clay is a promising application (Waajen et al. 2016). Also, in a series of articles, the possibility of application of calcium hydroxide or oxide for reservoirs with high pH and initially high water hardness was noted (Lürling et al. 2016).

In the case of shallow urban reservoirs, the bottom cleaning is one of the most common and expensive methods today. The removal of the seabed also removes its organic part including the substances of biological origin, which is one of the biggest phosphorus sources in the reservoir.

Precipitating reagents are now commonly used in water treatment and wastewater treatment. In addition, the use of polymer flocculants is a promising and inexpensive method of collecting biomass of phototrophic microorganisms (Gorin et al. 2015). The following precipitating reagents are used now:

1. Industrial flocculants based on polyacrylamide, polyethylene oxide, chitosan, etc.;
2. Flocculated biomass as ballistic agent (Gorin et al. 2015);
3. Ballast of quartz sand, dried soil or other mineral powders (Wang et al. 2016).

Type 1 reagents tend to be more effective at biomass precipitation but they are expensive, and besides, some of them may cause negative effects on ecosystems. Type 2 suggests using flocculated biomass of phototrophic microorganisms together with flocculating reagents, thus reducing their emission to the reservoir. Type 3 reagents are the safest, but they are the least effective.

During the use of some precipitating reagents, the biomass precipitates to the bottom of the reservoir and compacts with the layer of bottom sediments, which prevents further growth. A significant disadvantage of this method is that after sedimentation, the biomass cannot be processed further. However, sedimentation agents can be used in combination with other water body overgrowth control methods. For example, using precipitating reagents in combination with geotextiles can improve the efficiency of the biomass collection. For small ponds, partial pumping of water into a ballast tank for reagent treatment is possible.

Solutions based on membrane processes are also studied. In particular, the combination of coagulation/flocculation with ultrafiltration allows not only the collection of biomass, but also the removal of extracellular microcystine from water (Şengil et al. 2018).
Biofloculation is the use of different microalgae for biomass flocculation in water bodies. This method does not involve the use of chemical flocculants and can be used as a preliminary stage for chemical flocculation or as a separate method (Lee et al. 2009). The results of studies show that the addition of autoflocculating microalgae induces faster deposition of biomass and thus can improve the efficiency of biomass collection.

The effectiveness of combinations of methods of flocculation, sedimentation and biofiltration (FSBF) in laboratory conditions is shown using water collected from Chaohu Lake (China). When comparing this method to the method of water treatment with the help of sand filters, FSBF proved to be more effective in removal of microorganisms and some contaminants (Xu et al. 2017).

In a research on the effects of various flocculants for algae removal in Dianchi Lake (China), a polymeric iron sulphate-based flocculant was found to be the most effective. The researchers characterized this inorganic flocculant as efficient, safe and economical (Ma et al. 2015).

Chitosan and its compounds are effective flocculants capable of rapidly precipitating biomass to the bottom as well as disrupting microbial activity. To restore several degraded shallow lakes at once, the technology of ‘modified local soil ecological restoration’ has been developed (MLS-IER). The use of chitosan modified soil has proven very effective for the recovery of small eutrophic lakes (Pan et al. 2011).

Polyacrylamide is known as a well-functioning flocculant, but its use in sedimentation of biomass to the bottom of a reservoir raises some concerns. After sedimentation, polyacrylamide decomposes into acrylamides that are toxic to water body inhabitants. Acrylamides can be transferred to surface and ground waters (Guezennec et al. 2015). New modified polyacrylamides with declared reduced toxicity and high efficiency have been produced, but they have not yet been actively used.

The new types of flocculants are now being actively developed. Due to the low toxicity and proven effectiveness on model objects, polyethylene oxide flocculant is promising. The possibility of reducing the cost of the flocculation method by flocculated biomass recycling as ballast was shown. Reducing the amount of flocculants used will not only save money, but also reduce the possible negative impact of flocculants and coagulants on the water body (Gorin et al. 2015).

Another possible way to collect the biomass of phototrophic microorganisms is to use fibrous material. At work (Grigoriev et al. 2020) the possibility of efficient collection of Scenedesmus genus is shown using composite materials based on CPVC (JSC «Sterlitamak Petrochemical Plant») with active chitosan filler.

This work is aimed at investigating the effectiveness of flocculation of communities of phototropic microorganisms using various flocculants, their mixtures, coagulants and flocculated biomass as a ballast agent. In October, 2017 an algal bloom occurred in Pond Chernoistochinsky. Both the concentration of microorganisms and the type of microorganisms during this algal bloom were characteristic for this area. For example, similar algal blooms occurred in the Shershnevskoye reservoir which is the main water supply for the city of Chelyabinsk. In both cases Chlorophyta was the main dominant during the blooms (Kostryukova et al. 2019). To compare the efficiency of biomass flocculation, commercial flocculants containing polyacrylamide and polyethylene oxide often used for water purification were selected. Pond Chernoistochinsky was chosen as a typical reservoir of central part of Russia which suffers from eutrophication, as well as other water bodies in the area heavily loaded by various industrial enterprises (Namsaraev et al. 2020). If the effectiveness of flocculated biomass as a ballast agent will be demonstrated on a real lake water, it may become a serious competitor for all currently used flocculants.

**MATERIALS AND METHODS**

**Enrichment cultures and cultivation conditions**

Water samples from Pond Chernoistochinsky (coordinates N 57°43’, E 59°51’) located near Nizhny Tagil city in the Chelyabinsk region, Russia, were collected on October, 2017 in sterile plastic containers and immediately brought to laboratory for analysis. The enrichment culture was grown on modified BG-11 medium consisting of (g/L of distilled water): NaNO₃, 1.5; KH₂PO₄, 0.25; MgSO₄·7H₂O, 0.7; CaCl₂, 0.05; FeSO₄·7H₂O, 0.006; EDTA·2Na, 0.001; Na₂CO₃, 0.02; H₂BO₃, 0.00286; MnCl₂·4H₂O, 0.00181; ZnSO₄·7H₂O, 0.00022; Na₂MoO₄·2H₂O, 0.0004; CuSO₄·5H₂O, 0.00008; Co(NO₃)₂·7H₂O, 0.00005. Inoculum for experiments was cultivated in 500 mL Erlenmeyer flasks for 7 days (the stirring was provided by the orbital shaker with 180 rpm) and then in 3,000 mL flasks (the stirring was provided by the atmospheric air bubbling) for 7 days on the same medium. The growth conditions were kept constant under controlled temperature (21 ± 1 °C), with light intensity at 1,000 lux at all times during the process. The enrichment culture was harvested in the end of log phase and then used for flocculation efficiency tests.
Microscopy and morphotype quantification

Samples of water were analyzed using Nikon E200 microscope. The identification of cyanobacteria and green algae was performed using manuals (Komarek & Anagnostidis 2007) and (Dedusenko-Shchegoleva et al. 1959). The biomass of algae and cyanobacteria was determined using the volume counting method (Bryanskaya et al. 2006). For this purpose, the average volumes of the cells of each morphotype were calculated by equating them with simple geometrical figures. For unicellular organisms, cell numbers were counted. For filamentous organisms, total filament lengths were divided by the respective mean cell lengths to calculate cell numbers. Final cell numbers or total filament lengths were converted into biovolumes using geometric formulae (Sun & Liu 2003).

Flocculation experiments

Description of the flocculants

PAA-based flocculants non-ionic Superfloc N-300, anionic Superfloc A-100, cationic Superfloc C-492 (all three manufactured by KEMIRA), PEO-based flocculant cationic Sibfloc-718 (manufactured by Environmental Chemistry Research and Production Company, Russian Federation), and an inorganic coagulant FeCl₃*6H₂O (Komponent-Reaktiv Company, Russian Federation) were used.

Experimental design

The flocculation experiments on enrichment culture were carried out in 250 mL Erlenmeyer flasks. The single flocculant, flocculant mixtures or flocculated biomass were added in varied concentrations. After the addition of reagents, the test sample was stirred during 3 min and then left for sedimentation at room temperature. A control sample for each experiment contained no flocculant. All experiments were performed in triplicate. Experiments on flocculation had been carrying out for 120 min according to (Chen et al. 2013; Gorin et al. 2015).

Experiments with individual flocculant. The concentrations of individual flocculants were: Superfloc N-300, Superfloc A-100, Superfloc C-492 and Sibfloc ~718–25 mg/L, FeCl₃*6H₂O ~ 50 mg/L. The concentrations of coagulant and flocculants were chosen according to paper (Gorin et al. 2015).

Experiments with flocculants mixture. The flocculant mixtures consisted of ferric chloride (10; 15; 20; 50 mg/L) and Sibfloc-718 (2.5; 5; 10 mg/L).

Flocculated biomass experiments with flocculant mixtures. The coagulant and flocculant were added in the following concentration: ferric chloride ~ 15 mg/L + Sibfloc-718 ~2.5 mg/L. The material was stirred at 200 rpm during 3 min before it was left to settle for 120 min at room temperature, and then stirred again. The mixtures consisting of ferric chloride (15; 20 mg/L) with each of Sibfloc-718 (2.5; 1.25 mg/L) were added to reach 1.7% of volume of enrichment culture.

Flocculation efficiency evaluation

At certain intervals an aliquot was collected and optical density (OD) was measured using Thermo Scientific Genesys 10S UV-Vis spectrophotometer. The difference of OD values at 680 and 720 nm wavelength was registered. The flocculation efficiency was calculated by equation (Gorin et al. 2015): flocculation efficiency (%) = (1 – A/B) * 100, where A is OD of microalgal suspension after addition of flocculant, and B is OD control (without flocculant) at sedimentation time.

RESULTS AND DISCUSSION

Biomass and cell numbers of phototrophic microorganisms

The total biomass of the phototrophic microorganisms in the enrichment culture from the Chernoistochinsky Pond used for experiment was 29*10^-3 g/L (dry weight). The cell number reached 337*10^6 cells/L. The culture was dominated by unicellular green algae belonging to Acutodesmus genus (91.8% of the total biomass and 93% of the total number of cells). The amount of Scenedesmus did not exceed 3.4% of the total biomass and 3% of the total number of cells. Green unicellular cells probably belonging to different morphotypes of Chlorella comprised 2.1 and 1.7% of the total biomass. Other types of phototrophic microorganisms including one morphotype of filamentous cyanobacteria with thin 1 μm wide trichome didn’t exceed 1% of the total number of other phototrophic microorganisms.
Effect of different types and dosages of flocculants on flocculation efficiency in enrichment culture

In order to determine the most effective flocculating agent for the sedimentation of biomass of the enrichment culture obtained from the Pond Chernoistochinsky, an experiment was carried out including the check of the effectiveness of several types of flocculating agents. Figure 1 shows that positively, neutrally and negatively charged flocculating agents (Superfloc C-492, Superfloc N-300 and Superfloc A-100) based on polyacrylamide did not precipitate the biomass in the enrichment culture. Sibfloc-718 based on polyethylene oxide did not precipitate the culture as well. Ferric chloride was ineffective without the addition of polymeric flocculating agents, but in combination with a reagent based on polyethylene oxide it showed the best result.

As a result of the first experiment, it was found that the most effective of the tested variants of flocculating agents is PEO in combination with FeCl₃. More than 96% of biomass was sedimented in 120 minutes and more than 90% was sedimented in the first 5 minutes. Therefore, less reagents are needed for effective sedimentation of biomass. Thus, the aim of the next stage of the experiments was to identify the minimal concentration of flocculation agents FeCl₃ and Sibfloc-718 providing an efficient sedimentation of biomass.

Effect of coagulation/flocculation on flocculation efficiency in enrichment culture

In this experiment, the concentration of FeCl₃ was reduced from 50 to 10–30 mg/L. The concentration of Sibfloc-718 was reduced from 25 to 2.5–10 mg/L.

The addition of ferric chloride in concentrations of 10 and 15 mg/L to the culture has been shown to be ineffective. When these amounts were combined with PEO in concentrations of 10 mg/L, the degree of sedimentation did not exceed 30% (Figure 2(a)), and when combined with PEO in concentrations of 5 and 2.5 mg/L (Figure 2(b) and 2(c)), the culture precipitated but the forming flakes were extremely unstable. When combined with Sibfloc-718 and FeCl₃ at 2.5 and 20 mg/L, respectively, the flakes were also unstable. As a result of the preliminary experiment, the approximate coagulation threshold for the combination of PEO and FeCl₃ is 2.5 and 30 mg/L, respectively, leading to flocculation efficiency of 95.5%.

Effect of ballast agent on flocculation efficiency of enrichment culture

Based on the results obtained, a lower concentration of reagents was used in the next experiment devoted to the further reducing of the concentration of the flocculants used by adding the already flocculated biomass of the enrichment culture. For this experiment, biomass precipitated on the bottom as a result of adding of a ferric chloride and PEO reagents in concentrations of 15 and 2.5 mg/L mixture was used.

It was shown that when the ballast was added to the medium in the amount of 1.7% of the volume of the flocculated suspension, the coagulation threshold for the combination of PEO and ferric chloride was 1.25 and 15 mg/L, respectively (Figure 3). At FeCl₃ concentrations below 15 mg/L, the flocculated biomass flakes were extremely unstable. This effect is

![Figure 1](https://iwaponline.com/ws/article-pdf/21/8/4254/970392/ws021084254.pdf)
Figure 2 | Concentration of the enrichment culture from Pond Chernoistochinsky using flocculation agents FeCl₃ and Sibflocc-718 in the concentration range: (a) 10 mg/L Sibflocc-718 and 10–30 mg/L FeCl₃; (b) 5 mg/L Sibflocc-718 and 10–30 mg/L FeCl₃; (c) 2.5 mg/L Sibflocc-718 and 10–30 mg/L FeCl₃.
due to the fact that the flocculated biomass is already a suspension, which leads to a more rapid formation of aggregates from cells and their settling sedimentation. This method can help reduce the consumption of flocculants and, as a consequence, their negative impact on the state of water bodies.

The following conclusion can be made based on the data obtained. Cationic flocculants are more suitable for floculation of communities isolated from natural conditions. Combined use of a flocculant with a coagulant is even more effective. The greatest effect is possessed by the combined use of a flocculant with flocculated biomass. These data are consistent with the results obtained for monoculture (Gorin et al. 2015). These results can serve as a starting point for a more detailed study of the physicochemical properties of the cell surface in natural communities and their influence on sedimentation, in particular, zeta potential determinations.

The main advantages of the floculation method developed by us are the low toxicity of reagents and the possibility to use extremely low concentrations, which will impact not only the degree of influence on the pond and its inhabitants, but also reduce the cost of cleaning the pond from an excess biomass. The cost of floculation can be reduced by recycling flocculated biomass as ballast.

The method we have developed is promising for the control of eutrophication of reservoirs both on its own and in combination with other methods. The biomass flocculated using our method can be left to be degraded by the pond biota. Besides, our method can be a part of complex pond cleaning in the form of preliminary floculation of excess plankton biomass with subsequent mechanical bottom cleaning.

Our method can be applied both for small ponds and for the coastal shallow parts of large water reservoirs, which are most often subjected to harmful algae blooms. In this case, the application of the method will make it possible to stop eutrophication of a reservoir at its beginning without allowing the biomass accumulated near the shore to spread to deeper parts of the reservoir. We suppose that these results will find a wide application in controlling the results of eutrophication of water bodies in terms of the collection of biomass.

**CONCLUSIONS**

Our study showed that the combination of Sibfloc-718 and FeCl₃ reagents effectively precipitated the biomass of the enrichment culture from Pond Chernoistochinsky at the concentrations of 2.5 and 30 mg/L, respectively. Adding the reagents together with the flocculated biomass in the amount of 1.7% of the volume reduced the coagulation threshold by half.
Therefore, Sibfloc-718 floculant in combination with inorganic floculant FeCl₃ and a new sedimentation method with the addition of flocculated biomass may become a promising method of biomass harvesting and concentration in water bodies.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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