



Received 28.02.2019
Reviewed 02.04.2019
Accepted 24.04.2019

A – study design
B – data collection
C – statistical analysis
D – data interpretation
E – manuscript preparation
F – literature search

Experimental investigation of *Microcystis aeruginosa* cyanobacteria thickening to obtain a biomass for the energy production

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For citation: Malovany M., Zhuk V., Nykyforov V., Bordun I., Balandiukh I., Leskiv G. 2019. Experimental investigation of *Microcystis aeruginosa* cyanobacteria thickening to obtain a biomass for the energy production. Journal of Water and Land Development. No. 43 (X–XII) p. 113–119. DOI: 10.2478/jwld-2019-0069.

Abstract

The purpose of the presented research is to analyse possible methods of thickening of the *Microcystis aeruginosa* (Kützing) Kützing cyanobacteria using the obtained concentrate as a biomass for the production of energy carriers and biologically valuable substances. Method of cyanobacteria thickening under the action of electric current and in the electric field, as well as the method of coagulation–flocculation and gravity thickening, was experimentally investigated in lab-scale conditions. Electrical methods didn't show positive results for the *Microcystis aeruginosa* thickening, despite the reports of their potential efficiency in a number of previous studies. The high efficiency of the method of coagulation–flocculation and gravity thickening of *Microcystis aeruginosa* suspensions was obtained. The optimum concentrations of industrial polymeric coagulants and flocculants for the thickening of *Microcystis aeruginosa* suspensions were defined in the range of about 10 ppm for the coagulants and about 1 ppm for the flocculants. Negative effect of the previous cavitation treatment of the diluted suspensions of *Microcystis aeruginosa* on the effectiveness of the coagulation–flocculation and gravitational thickening was confirmed experimentally. Hydrodynamic cavitation should be recommended to use after the thickening as the next step of processing of concentrated suspensions of *Microcystis aeruginosa* to achieve maximum extraction of energy carriers and biologically valuable substances.

Key words: coagulation–flocculation, cyanobacteria, electric field, energy carriers, gravity sedimentation, suspension, thickening

INTRODUCTION

The results of unsustainable anthropogenic activities are the pollution of all components of the environment:

atmosphere, hydrosphere and lithosphere. The methods of the monitoring of the hydrosphere pollution and innovative treatment technologies are still important topics of the scientific research during the last decade. In particular, in

Ukraine the extensive study of sewage treatment using ion-exchange technologies was carried out by GOMELEYA *et al.* [2016]; treatment using different types of cavitation process was investigated by STARCHEVSKYY *et al.* [2017] and KOVAL *et al.* [2011]; using of reagent methods – by GOMELEYA *et al.* [2014]; using of electrical extraction – by GOMELEYA *et al.* [2018]; monitoring of the hydrosphere pollution by the mine waste waters was studied by the PAVLICHENKO and KROIK [2013] and KULIKOVA, PAVLYCHENKO [2016]; analysis of the environmental hazards from the uncontrolled growth of blue-green algae and suggestions for the minimization of resulting risks are investigated by MALOVANYI *et al.* [2016], etc.

New results of experimental studies of the blue-green algae thickening for the subsequent use of thickened biomass for the production of renewable energy carriers are presented in this work. Thickening was realized using two different methods: coagulation-flocculation process and the action of electric field or electric current.

The production of biofuels using algae as raw material is a promising way of obtaining the renewable energy sources that is the basis of sustainable development. Natural algae suspensions usually have a low organic content – from 0.5 g up to 4.0 g of dry matter per 1 dm³ [CHATUNGNOEN, CHISTI 2016]. For the efficient processing, these suspensions need to be thickened.

Basic methods of algae suspensions thickening are as follows: sedimentation, filtration and microfiltration, centrifugal dewatering, flotation, electrocoagulation, direct osmosis, and others. The feature of the cyanobacteria suspensions is that the specific mass of the cells almost the same as the specific mass of water. This fact virtually eliminates the non-reagent sedimentation, as well as significantly decreases the efficiency of centrifugation.

In the patent of TOPACHEVSKYI *et al.* [1969] a method of thickening of the blue-green algae water suspensions, using the electric current was invented. The high efficiency of the proposed method for the algae thickening and harvesting on the anode was obtained by the authors. This method of algae thickening was tested in the study.

The method of coagulation and flocculation deposition is a simple and sufficiently effective method for thickening of low-concentrated suspensions in natural waters [GERCHMAN *et al.* 2017; GRIMA *et al.* 2003; MALCZEWSKA 2016; MALCZEWSKA, BICZYŃSKI 2017; VANDAMME *et al.* 2013]. The analysis of the results of coagulation-flocculation thickening of cyanobacteria suspensions indicates that the technical efficiency of such treatment strongly depends on: 1) the type of cyanobacteria and the chemical composition of the aqueous suspension [CHATUNGNOEN, CHISTI 2016; DAS *et al.* 2016; GERDE *et al.* 2014]; 2) the initial concentration of cells in the suspension and the temperature conditions of the process [GERDE *et al.* 2014; GRANADOS *et al.* 2012; LAAMANEN *et al.* 2016]. A large variety of species characteristics of cyanobacteria and a wide range of the physicochemical characteristics of suspensions make it necessary to select reagents for a particular species of algae and taking into account the initial concentration of the organic matter in the suspension.

The next stage of the treatment after the thickening of the cyanobacteria suspensions as usual is rigid action on thickened biomass in order to destroy the algae cells. One of the promising ways of cell membranes destroying, which increases the efficiency of extraction of energy sources and biologically valuable substances from cyanobacteria, is ultrasonic cavitation [CHENG *et al.* 2014; GREENLY, TESTER 2015]. The results indicate that the effectiveness of cavitation treatment depends mainly on the type of algae and the strength of their cell membranes. The effectiveness of cavitation destruction of algae cells increases rapidly at the beginning of treatment (in the first 20–30 s), after which the process is much slower. The possible explanation for this effect is the extinction of ultrasonic energy in the cloud of cavitation bubbles, which is formed during the processing [GREENLY, TESTER 2015]. If the concentration of cells in the suspension increases, the effectiveness of the cavitation treatment is somewhat diminished, but the cost-correlation ratio for the high-concentrated suspensions seems to be more economically feasible. For example, ultrasound treatment of *Isochrysis galbana* Parke suspension with a mass concentration of cells of 5000 ppm using the ultrasound with the frequency of 20 kHz, energy density 2.5 W·cm⁻³ and processing time of 25 s resulted in 86.1% cell destruction, whereas for a concentration of 30000 ppm and $t = 45$ s – 88.9%, and for concentration of 75000 ppm and $t = 40$ s – 73.6% [GREENLY, TESTER 2015]. The cavitation treatment of the cyanobacteria suspensions can be effective also for selective impacts on the biomass. For example, an effective selective action of the cavitation treatment of cyanobacteria to remove the spinal cord from the suspension is demonstrated [KIM *et al.* 2017].

Thus, the thickening of cyanobacteria suspensions, mechanically gathered in natural or artificial conditions, is a necessary initial stage of their treatment. Analysis of the results of studies on the algae suspensions thickening indicates that one of the most effective modes of thickening remains the gravitational sedimentation after the coagulation-flocculation pretreatment. A large variety of the characteristics of cyanobacteria species and a wide range of physicochemical parameters of suspensions make it necessary to select reagents for a particular algae species and even for the initial content of organic matter in the suspension. Important parameters of the thickening process are the temperature and pH value of medium. The optimal values of these parameters are also variable depending on the type of algae.

In the Ukrainian climatic conditions the *Microcystis aeruginosa* (Kützing) Kützing cyanobacteria can be effectively used for both energy substances and biologically valuable materials. *Microcystis aeruginosa* is a species of freshwater cyanobacteria, which causes the eutrophication of water bodies, which is harmful from both ecological and economic points of view. *Microcystis aeruginosa* have small sized cells (up to several micrometers in diameter) without shells. Cyanobacteria produce neurotoxins and peptide hepatotoxins such as microcystin and cyanopeptoline, which are the threat both to the water body biocenosis and to the people if water is used for the domestic water

supply. *Microcystis aeruginosa* can be an effective source of butyl-hydroxytoluene – an antioxidant, nutritional supplement and industrial reagent [BABU, WU 2008]. Hence, the gathering of the *Microcystis aeruginosa* in natural water bodies and their use for the extraction of biologically valuable substances and for the obtaining sustainable energy sources has a triple potential benefit: decreasing of environmental pollution, minimization of economic losses for water utilities, and obtaining the renewable energy sources and biologically valuable substances.

MATERIALS AND METHODS

The object of the study is the aqueous suspensions of freshwater cyanobacteria *Microcystis aeruginosa* with an initial content of algae cells in range from 200 ppm to 1000 ppm (mass concentration by dry matter).

The method of concentrating the aqueous suspension of blue-green algae using an electric current was verified at experimental installation consisting of a glass 150 cm³ flask with two immersed vertically placed copper electrodes, each with a surface area of 10 cm². The distance between the electrodes is 25 mm. The power source is the voltage regulator PSIP-500, and the current value is controlled with the help of an ammeter M2038. To eliminate the effect of dissolved ions, the suspension of blue-green algae was made by diluting the concentrated algae mass in distilled water. In the course of the experiment, the 1 mm³ probes of suspension were carried out near the center of each electrode to determine the optical density of the medium. The optical density was measured using a spectrophotometer SF-46 with a wavelength of 450 nm, since blue-green algae have one of the absorption maxima in this range [MERZLYAK *et al.* 2008].

The method of thickening of the aqueous suspensions of *Microcystis aeruginosa* using the coagulation–flocculation pretreatment was studied using the industrial coagulants and flocculants. Such reagents were tested:

- polymeric aluminum coagulants PAX-18 and PAX-XL19H,
- flocculants A100 and C494.

Based on the qualitative results of preliminary studies, flocculant C494 was eliminated from the consideration, so as it requires subsequent filtration or microfiltration of the suspension.

In the investigated suspension of *Microcystis aeruginosa* (without cavitation treatment, or after cavitation treatment), an appropriate reagent composition was added. Content of additives in different samples of suspension are given in Table 1.

In order to improve the techno-economic indicators of the method researches were done using the very diluted suspensions (Tab. 1). The mass contents of PAX-18 and PAX-XL19H coagulants in different suspensions were equal 10 ppm or 1 ppm, and the mass content of flocculant A100 was 10 ppm or 1 ppm, if it used separately, and only 1 ppm if it used in combination with coagulants.

The kinetics of the lab-scale thickening process of *Microcystis aeruginosa* was investigated on the suspensions

Table 1. Content of additives in the study of *Microcystis aeruginosa* (Kützing) Kützing aqueous suspensions thickening by sedimentation with coagulation–flocculation pretreatment

No. of suspension sample	Mass content of additives (ppm)		
	PAX-18	PAX-XL19H	A100
1	–	–	–
2	10	–	–
3	1	–	–
4	–	10	–
5	–	1	–
6	–	–	10
7	–	–	1
8	10	–	–
9	–	10	1

Source: own elaboration.

with initial mass cell concentration of $C_0 = 500$ ppm by dry matter.

The influence of hydrodynamic cavitation on the process of *Microcystis aeruginosa* thickening was studied on the experimental lab-scale installation with a dynamic type cavitator (Fig. 1). The study of the process of microbiological contaminants destruction was performed on this installation.

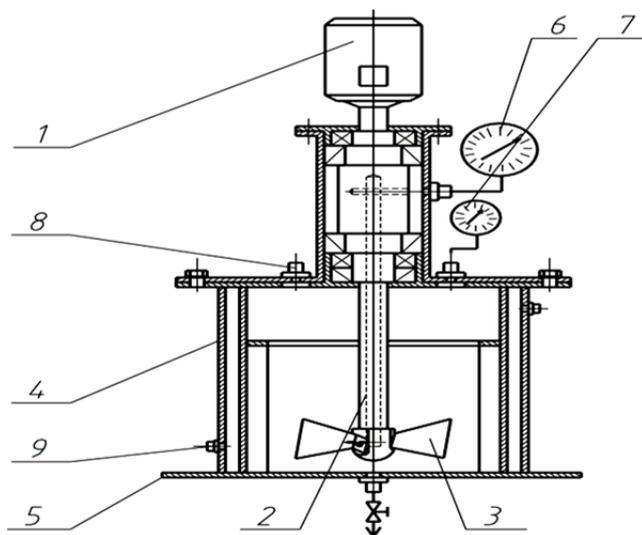


Fig. 1. Scheme of the laboratory-experimental installation: 1 = electric motor, 2 = shaft, 3 = cavitation device, 4 = tank, 5 = frame, 6 = tachometer, 7 = gauge, 8 = samplers, 9 = connection for the coolant supply; source: own elaboration

The installation consists of an electric motor, a tank with volume of 1.5 dm³, made from organic glass, a cavitation device, attached to a hollow shaft. All the installation is fixed on the frame. A three-bladed stirrer of wedge-shaped profile with acute anterior and blunt rear edges was used as a cavitation device. Tank was filled with 1.0 dm³ of tested suspension. The effect of cavitation pretreatment was studied in conditions of cavitation mixing with the rotational speed of 4000 rpm and duration of 15 min. After cavitation pretreatment, the sample was subsequently used to study the thickening effectiveness of the microalgal suspension.

RESULTS AND DISCUSSION

EFFECT OF ELECTRIC CURRENT AND ELECTRIC FIELD ON THE CYANOBACTERIA SUSPENSIONS

The study of the effect of electric current on the aqueous suspension of the cyanobacteria showed that, under the conditions specified by TOPACHEVSKYI *et al.* [1969], the concentration of algae on the electrodes practically does not occur. Since the water composition is different in both experiments, the current density between the electrodes was taken into account. However, in this point of view there was no visible effect. The visual thickening of the cyanobacteria at the same observation periods as in paper by TOPACHEVSKYI *et al.* [1969] occurs at a current density of $0.008 \text{ A}\cdot\text{cm}^{-2}$, which is one order of magnitude higher than reported by TOPACHEVSKYI *et al.* [1969]. At the same time there is an emission of hydrogen on the cathode that confirms the electrolysis of water. The dependences of the optical density of the suspension near the electrodes from the time of electric current action are shown in Figure 2.

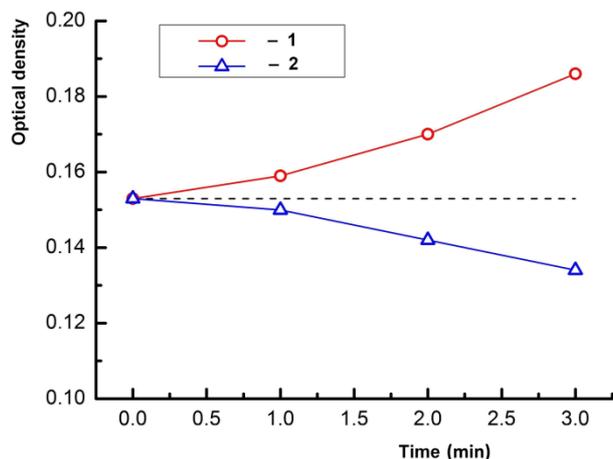


Fig. 2. Dependences of the optical density of the suspension near the electrodes from the time of electric current action with current density of $0.008 \text{ A}\cdot\text{cm}^{-2}$: 1 = anode; 2 = cathode; the dashed line means the initial value of the optical density of the suspension; source: own study

A small increase of optical density of suspension near the anode was observed. Instead, on the cathode optical density was slightly decreasing (Fig. 2). The first thing is due to the thickening of the algae, and the second – the result of the algae content decreasing and of the hydrogen emission on the cathode, which facilitates the mixing of the suspension and the algal migration to the surface. Sampling of the biomass near the electrodes was carried out 3 min after the experiment began, because longer time of electrolysis caused forming on the anode surface dense deposits of algae, which did not allow to do a proper sampling.

After 5 min of electrolysis, the electrodes were switched off from the power supply, taken out and photographed. The anode clearly shows the thickened layer of algae, despite the fact that some of them were washed away when removing the electrode from the suspension; the cathode is free from algae at all (Photo 1).

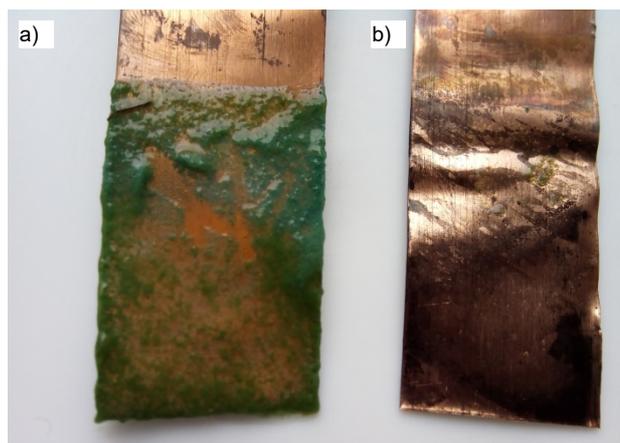


Photo 1. Electrodes after the action of electric current on the suspension: a) anode, b) cathode (phot. I. Bordun)

At the next stage of the experiment the effect of the electric field on blue-green algae without the electric current through the suspension was studied. For this purpose, a cylindrical capacitor was assembled. The inner electrode of capacitor was made from 5 mm diameter stainless steel, and external electrode was a hollow cylinder made of steel foil. The suspension was poured into a glass vessel. Inner electrode was placed inside the vessel, and external electrode of the capacitor was fixed outside the vessel. The gap between the electrodes was 30 mm. The capacitor was supplied with voltage from the high-voltage stabilizer 807 R (Mauler Nukleonic). However, even at voltage of 7 kV between the electrodes, no visible thickening of algae occurred.

Thus, experimental lab-scale studies of the blue-green algae thickening in electric field showed the following: 1) blue-green algae can be thickened using an electric current through the suspension, but this process requires significant energy consumption and is complicated by electrolysis of water; 2) the thickening of algae occurs at the anode surface; 3) action of an electric field on the aqueous suspensions of blue-green algae does not caused any thickening of algae near the electrodes.

EFFECT OF GRAVITATIONAL SEDIMENTATION AFTER THE COAGULATION-FLOCCULATION PRETREATMENT OF THE CYANOBACTERIA SUSPENSIONS

The results of cyanobacteria suspensions thickening by sedimentation with preliminary coagulation and flocculation treatment with the PAX-18 and PAX-XL19H polymer coagulants, as well as the A100 flocculant are shown in Photo 2. The numbers of the measuring cylinders correspond to the type and concentrations of the additives indicated above in the Table 1.

The height of the thickened layers of cyanobacteria suspensions after 24 h of sedimentation are presented in Figure 3.

Final results, presented in Figure 3, do not allow directly choosing the optimal composition of additives. The analysis of changes in time of main treatment parameters was performed, taking into account the kinetics of clarify-

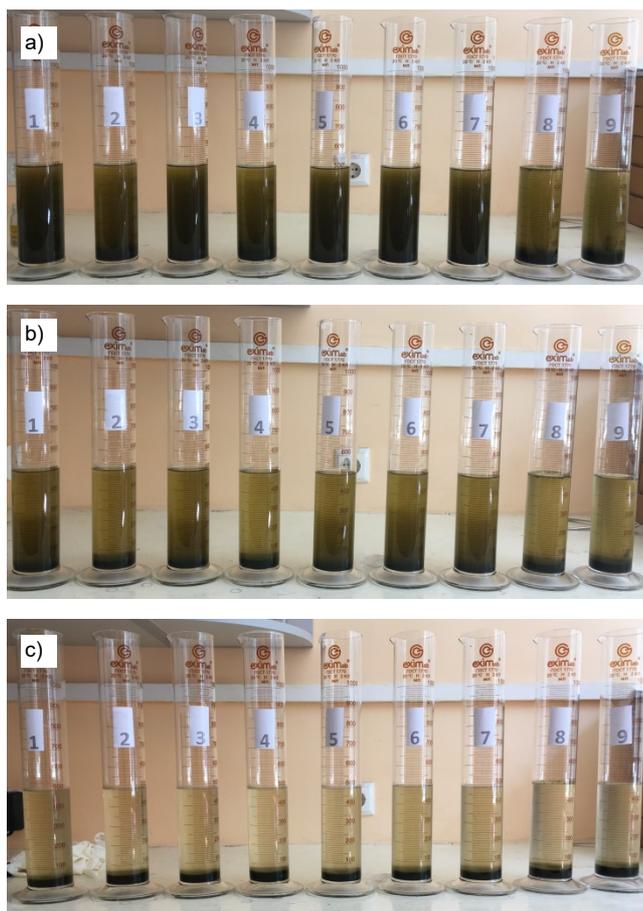


Photo 2. Kinetics of sedimentation of *Microcystis aeruginosa* suspensions in lab-scale conditions: a) after 5 min; b) after 30 min; c) after 24 h; 1–9 no. of samples as in Tab. 1 (phot. I. Balandiukh)

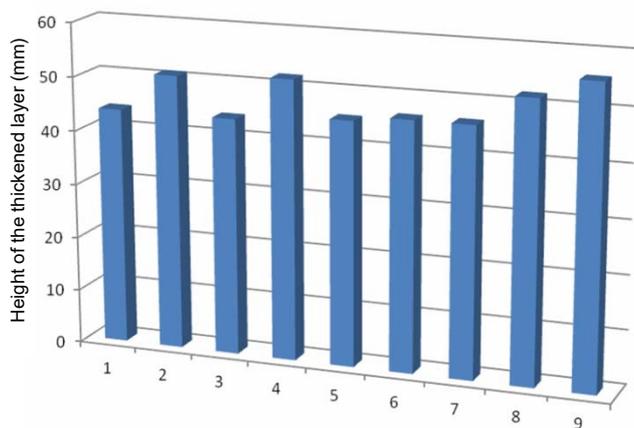


Fig. 3. The values of the height of the thickened layers of cyanobacteria suspensions after 24 hours of sedimentation for the samples; 1–9 = no. of samples as in Tab. 1; source: own study

cation of the suspensions and kinetics of the sedimentation layer growing.

Based on this analysis, the optimum results of thickening (by the ratio of effect – costs) were obtained while simultaneously adding polymeric coagulant and a flocculant A100 to the suspension. Using the PAX-18 and PAX-XL19H coagulants with mass concentration 10 ppm and

flocculant A100 with concentration 1 ppm allowed to get within 30 min of sedimentation the effect of suspensions thickening about 11.8 and 10.4 times in volume, respectively. The mass content of *Microcystis aeruginosa* cells in the thickened biomass as a result of coagulation–flocculation pretreatment and sedimentation increased by 9.6 and 9.0 times, respectively, up to 4800 ppm and 4500 ppm, respectively.

On the other hand, cavitation pretreatment of algae suspensions decreases the effect of their thickening. For all compositions of additives in Table 1, the negative influence of the previous cavitation treatment of diluted suspensions of *Microcystis aeruginosa* on the effectiveness of their subsequent gravitational thickening was experimentally obtained. No significant effect of the separation of these mixtures was obtained even after 24 h of precipitation.

CONCLUSIONS

Lab-scale studies of the cyanobacteria suspensions thickening using the electric field has not shown positive results, despite the available previous information on its possible effectiveness.

Study of the electric current effect on the cyanobacteria suspensions verified that some thickening of algae occurs at the anode surface, but this process requires significant energy consumption and is complicated by electrolysis of water.

The high efficiency of the method of coagulation–flocculation and gravity thickening of suspensions was observed in lab-scale experiments with freshwater cyanobacteria of the species *Microcystis aeruginosa*. Aqueous solutions of polymer-aluminum coagulants PAX-18 and PAX-XL19H, as well as flocculants A100 and C494, were used for preliminary coagulation–flocculation processing of suspensions.

The optimum concentrations of coagulants and flocculants for the thickening of *Microcystis aeruginosa* suspensions in laboratory conditions were defined. The most effective thickening of the *Microcystis aeruginosa* suspensions with initial cell content 500 ppm (by dry matter) was obtained while simultaneously adding to the suspension polymeric coagulant PAX-18 or PAX-XL19H and flocculant A100. Using the PAX-18 and PAX-XL19H coagulants with mass concentration 10 ppm and flocculant A100 with concentration 1 ppm allowed to get within 30 min of sedimentation the effect of thickening about 11.8 and 10.4 times in volume, respectively. The mass content of *Microcystis aeruginosa* cells in the thickened biomass as a result of coagulation–flocculation pretreatment and sedimentation increased by 9.6 and 9.0 times, respectively, up to 4800 ppm and 4500 ppm, respectively.

Negative effect of the previous cavitation treatment of the diluted suspensions of *Microcystis aeruginosa* on the effectiveness of the coagulation–flocculation and gravity thickening was confirmed experimentally. Hydrodynamic cavitation should be recommended to use after the thickening as the next step of processing of concentrated suspensions of *Microcystis aeruginosa* to achieve maximum extraction of energy and biologically valuable substances.

ACKNOWLEDGEMENTS

Publication supported by the Polish Ministry of Science and Higher Education as a part of the program of activities disseminating science from the project "Organization of the First International Science Conference – Ecological and Environmental Engineering", 26–29 June 2018, Kraków.

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**Eksperymentalne badania nad zagęszczaniem cyjanobakterii *Microcystis aeruginosa*
w celu uzyskania biomasy do produkcji energii**

STRESZCZENIE

Celem przedstawionych badań była analiza możliwych metod zagęszczania cyjanobakterii *Microcystis aeruginosa* (Kützing) Kützing do uzyskania koncentratu biomasy przydatnego do produkcji energii i substancji czynnych biologicznie. W skali laboratoryjnej analizowano metody zagęszczania pod wpływem prądu i pola elektrycznego oraz metody koagulacji–flokulacji i zagęszczania grawitacyjnego. Metody elektryczne nie dały pozytywnych wyników mimo wielu wcześniejszych badań na temat ich potencjalnej efektywności. Dużą efektywność uzyskano natomiast w przypadku metod koagulacji–flokulacji i grawitacyjnego zagęszczania zawiesiny *Microcystis aeruginosa*. Optymalne stężenie przemysłowych polimerowych koagulantów użytych do zagęszczania ustalono na 10 ppm, a flokulantów – na 1 ppm. Doświadczalnie potwierdzono ujemny wpływ wcześniejszego poddawania rozcieńczonych roztworów *Microcystis aeruginosa* działaniu kawitacji na skuteczność zagęszczania metodami koagulacji i flokulacji oraz zagęszczania grawitacyjnego. Hydrodynamiczną kawitację zaleca się stosować po zagęszczeniu, jako następny etap w przetwarzaniu zagęszczonej zawiesiny *Microcystis aeruginosa* do postaci nośnika energii i pozyskania substancji biologicznie czynnych.

Słowa kluczowe: cyjanobakterie, koagulacja-flokulacja, nośnik energii, pole elektryczne, sedymentacja grawitacyjna, zagęszczanie, zawiesina