The effects of barley straw extract on the microalgae growth

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Abstract. One of the most common environmental problems of recent decades is the eutrophication of surface water and the associated algal bloom. As one of the approaches to eliminate this phenomenon, the use of biological methods, in particular biological preparations from barley straw, is considered. In this work presents the results of laboratory modeling of the effect of 1 and 2\% (v/v) of barley straw (Hordeum vulgare L.) extract on the development of unicellular algae (Chlorella vulgaris Beijerinck). The allistic effect was evaluated by the parameters of algae growth, which were measured using the spectrophotometric and fluorescent methods. The parameters were measured at 0, 7, 14 and 21 days. The addition of barley straw extract reduced algae growth, both with the addition of 1\% and 2\% concentration. A two-day toxicity test using Daphnia magna Straus showed no toxic effect of barley straw extract for both 1\% and 2\% concentration.

1. Introduction
Algal blooms in water bodies is a widespread problem. The massive development of algae causes deterioration of water quality – its color changes, an unpleasant smell appears. In the process of extinction and decomposition of algae, a significant amount of dissolved oxygen is absorbed, which leads to hypoxic conditions. An insufficient amount of dissolved oxygen in water can lead to the death of aquatic organisms. In addition, some species of blue-green algae can produce a variety of secondary metabolites – cyanotoxins, which have a detrimental effect on aquatic flora and fauna [1, 2, 3, 4, 5, 6].

Currently, many methods have been developed to prevent the eutrophication of water bodies and the blooming of algae: physical, chemical, biological. Available methods for reducing eutrophication vary widely depending on efficiency, cost, frequency of use and range of applicability. The biological methods of prevent the mass development of algae include measures on introduction of organisms or biological substances that release into the water allelopathic substances which in turn inhibit the growth of phytoplankton.

In this study we considered the biological method of prevention the mass production of algae. Using this method in different countries have demonstrated its effectiveness in reducing algal blooms without any undesirable consequences.

The method is because during the decomposition of barley straw, substances that inhibit the development of algae are released into the water – various phenolic compounds (decomposition products of lignin) and some other.

The literature describes experiments confirming the algistatic properties of barley straw, carried out both in laboratory conditions [7] and in field trials [8]. Straw was active at very low concentrations, inhibiting the growth of a number of algae, including unicellular and filamentous algae and...
cyanobacteria [9]. The potential of this method of controlling undesirable algae growth was confirmed by Newman and Barrett [10], who showed that decaying barley straw inhibited the growth of cyanobacterium Microcystis aeruginosa, which is known to release dangerous toxins into the water.

2. Material and methods

In this study, we evaluated the inhibitory effect of barley straw by its influence on the growth of the microalgae Chlorella vulgaris Beijerinck (Chlorophyta) in the laboratory modeling.

In the experiments, an extract from barley straw (Hordeum vulgare L.) was used. For its preparation, 10 g of dry barley straw were ground in a laboratory mill (the size of single fragments was on average 0.5–1 cm). 2.5 g of chopped straw and 250 ml of distilled water (Barnstead Pacific RO Water Purification System, Thermo Scientific, USA) were added to 500 ml conical flasks. Then the mixture was boiled for 2 hours on a hotplate. After that, the mixture was cooled and the solution was filtered through a filter paper, the filtrate volume was adjusted to 250 ml with distilled water (modification [11]). The extract was then stored at −20 °C until required.

The Chlorella vulgaris culture was grown on 50% Tamiya medium prepared from concentrated solutions of nutrient salts.

In further experiments, conical flasks of 500 ml were used. For the study was prepared for 12 flasks: six control flasks (control) and six flasks for the study of the extract (experiment). A chlorella culture (with an initial optical density of 0.150) was added to a 500 ml cylinder, was adjusted to the mark with distilled water, and then poured into conical flasks.

In the prepared samples, the initial spectral absorbance of the algae culture was measured on an IPS-03 instrument (Energolab, Russia) in cylindrical cells with a diameter of 2 cm at a wavelength \( \lambda = 560 \) nm, and the fluorescence level was measured on a Foton-10 instrument (Energolab, Russia). If necessary, the conversion of the spectral absorbance of the chlorella suspension to the concentration, expressed in the number of cells in one milliliter, was carried out according to the formula: \( N = kD \) where \( N \) is thousands of cells, \( k \) is the proportionality coefficient (obtained by us is 23 702; \( R^2 = 0.894 \)), \( D \) is spectral absorbance.

After the initial measurements were carried out, the previously prepared barley straw extract was added to the flasks in the quantities necessary to achieve 1 and 2% of the extract concentration (by volume), respectively.

To prevent the algae from settling and to provide the culture with carbon dioxide, aeration was carried out in continuous mode through tubes connected to an Air-001 air pump (Barbus, China) with an intensity of 500 ml/min per flask. As a result, the CO2 content in the culture medium was maintained at a constant level by dissolving the carbon dioxide contained in the air. During cultivation, the algae suspension was irradiated with a fluorescent lamp mounted above the flasks in a continuous mode.

To study the possible toxic effect of barley straw extracts (1 and 2%) an acute toxicity test with Daphnia magna Straus (Cladocera, Crustacea) was performed [12].

Statistical analysis was performed using STATISTICA 10 (StatSoft, USA).

3. Results and discussion

The algistatic effect was evaluated by measuring the spectral absorbance at 0, 7, 14, and 21 days under control and experimental conditions, respectively. Figure 1 shows the spectral absorbance with the addition of 5 ml of barley straw extract (1% v/v).

According to the presented data it is clear that by the end of the experiment (on day 21) the spectral absorbance in the flasks with the addition of barley straw extract was less than in the flasks without adding it – averaged 0.3 units. On days 7 and 14, the difference between experiment and control was 8.2 and 17.7%, respectively. For 21 days, the difference increased slightly, it was 18.3%.

Similarly, data on spectral absorbance in flasks with the addition of 10 ml of barley straw extract were analyzed (2% v/v concentration). The dynamics of changes in spectral absorbance is shown in figure 2.
According to the presented data it is clear that the spectral absorbance values of the suspension in flasks with the addition of barley straw extract by the end of the experiment were also lower than in flasks without addition of the extract, the average value for 21 days was 0.292. On day 7, the difference between experiment and control was 16.4%, which is 2 times more than in the experiment with the addition of 5 ml of barley straw extract.

On day 14, the experiment was less than control by 27.9%, which is also almost twice as much as in the experiment with a lower dose of the extract. However, on day 21, by the end of the study, the difference between experiment and control was 17.5%. This can probably be explained by the fact that the effectiveness of the barley straw extract decreases with time (after 14 days).

During the experiment, the phenomenon of “cell-cell adhesion” of algae was observed. Large flakes were found both in flasks with a 1% concentration of barley straw extract, and with a 2% concentration.

Bratby [13] writes that flocculation is a well-established method for removing suspended solids in the process of water purification. However, this method is slightly worse designed to remove microalgae. Their cells typically have a diameter of <15 μm and a density only slightly larger than that of water. As a result, algae cells weakly settle by gravity. In addition, as indicated Gerardo et al. [14], the cell surface is negatively charged due to ionized functional groups on the cell wall. Electrostatic
repulsion between cells prevents them from converging and spontaneously sticking to each other. In the process of flocculation, flocculants are used to neutralize the surface charge on the cells. Vandamme et al. [15] noted that since the 80s, tannin-based polymers have increasingly been used as flocculants. As mentioned above, plant phenolic compounds - tannins are a part of barley straw. In our study, the phenomenon of “cell-cell adhesion” of algae can also be explained by the presence of these substances.

To register a decrease in the number of algae cells, one can use the methods of counting cells under a microscope, as well as by measuring the fluorescence of algae chlorophyll. The values of fluorescence of chlorella in experiments with the addition of barley straw extract of 1% concentration relative to the control are presented in figure 3.

The values of the intensity of the fluorescence in the flasks with the addition of the extract were lower than in the flasks without the addition of 21 and 24% at 7 and 14 days, respectively.

In the experiment with the addition of barley straw extract of 2% concentration, the intensity indicators of the zero level of fast fluorescence in the experimental flasks on days 7 and 14 were lower than the control by 30 and 49% respectively (figure 4).
A prerequisite for the possible use of a barley straw extract in water bodies is its safety for aquatic animals. Using barley straw is an environmentally friendly method. There are no reports of negative consequences for aquatic invertebrates or fish in literary sources, except for cases when excessive amount of straw was used on small ponds, which led to oxygen depletion in the pond. These excessive doses were at least 100 times higher than the doses recommended by various researchers.

A two-day acute toxicity test using crustaceans (*Daphnia magna*) showed no toxic effect of barley straw extract for both 1% and 2% concentration. The toxicity index in both cases was 0%, i.e. daphnids felt the same way as in the standard cultivation water.

To eliminate algal bloom, barley straw extract can be applied from boat to surface waters by spraying or entering into a wake stream. The recommended frequency of application is 1 time per 2 weeks during the growing season (from the end of May to the end of August). The dosage of application depends on the volume of the reservoir/lake. For water bodies with an already existing problem of algal blooms, the initial amount of the extract is recommended to be increased. As a preventive measure, it is possible to add a small amount of the extract before the beginning of the mass development of algae.

4. Conclusion

The study showed that the extract obtained from barley straw, is quite effective for inhibiting the growth of algae and can be used to suppress their excessive development. It is believed that this method has no negative environmental consequences, and in general, the application of the considered biological method of preventing the development of algae is an effective and inexpensive method of eliminating algal blooms.

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