<u>Entry to the Stockholm Junior Water Prize 2019</u>

Ministry of Education of the Republic of Belarus
Educational Institution "Republican Center for Ecology and Local Study" (RCELS)

ADDING OF MICROALGAE CHLORELLA VULGARIS IMPROVE SOME BIOLOGICAL PARAMETERS OF FISHPOND

Authors:
Egor Sokol, student of the 11 class, Novoelnyanskaya high school, 17 years
Teacher: Bakovich Elena

Alexandra Khankevich, student of the 10 class, Borisov Center for Ecology and Tourism, 16 years
Teacher: Alena Shubara

Supervisor: Ivan Russkikh, head of laboratory of RCELS

BELARUS (Novoelnia, Borisov, Minsk)-2019

SUMMARY

Aquaculture, which in Belarus means breeding and growing mainly fish, is one of the most promising industries for low-volume private agriculture in Belarus. The study and promotion of aquaculture has initiated the active creation of many fish farms in recent years. The development of private fish farms is consistent with government goals and allows solving problems of ensuring the country's food security and import substitution of food products.

The mirror carp is one of the most valuable promising objects of fish farming in Belarus. Along with the temperature and feeding regime, protection from diseases and parasites, water quality is the most important factor contributing to the success of carp breeding.

On the other hand, breeding fish in ponds, in particular, mirror carp, leads to changes in water quality, resulting in eutrophication and subsequent pollution. In most cases, the ecological status of water bodies is closely related to human activities. The increase in the production of mirror carp in ponds of Belarus will inevitably lead to a deterioration in the ecological status of water bodies and related territories.

Much has been done to prevent pollution of water bodies both in research and in practice. However, a single mechanism capable of effectively protecting water bodies from both exogenous and endogenous pollution has not yet been developed. Most likely, the solution to this problem is an integrated approach to it. This is to achieve the biological rehabilitation of reservoirs used for aquaculture.

Biological rehabilitation of polluted reservoirs occurs through the natural processes of self-purification due to the vital activity of microorganisms, plants and animals inhabiting these reservoirs. However, this process takes quite a long time and its effectiveness is often low.

To speed up the processes and increase the efficiency of the recovery of polluted water, the use of the method of biological rehabilitation due to the algolization of the alga Chlorella vulgaris is possible.

In order to assess the effectiveness of using the algolization method to restore the quality of ponds, we conducted algolization with our own algae strains and estimated parameters such as the content of nitrates (mg/l), nitrites (mg/l) and ammonium (mg/l), phosphates (mg/l), iron (mg/l), biological oxygen demand (mg/l), acidity of water (pH), its smell, transparency and colour, surface water temperature (°C), level of water bloom, bacterial contamination of water (CFU/ml) and other. We also determined the effect of algolization on the productivity of mirror carp.

As a result, the obtained data indicating the high efficiency of algolization of fish ponds with the isolated chlorella strain, which was expressed both in improving the studied water parameters and in increasing the carp mirror productivity, thereby achieving an economic effect of algolization.

A study on the biological rehabilitation of the waters of fish ponds by algolization them with a strain of Chlorella vulgaris showed the possibility of recovering polluted waters. Compared to natural self-purification, biological rehabilitation is much more intensive, although a wide range of microorganisms, plants and animals inhabiting the reservoir take part in the self-purification process. This achieves environmental benefits and practical significance of the study.

TABLE OF CONTENTS

INTRODUCTION

Aquaculture, which in Belarus means breeding and growing mainly fish, is one of the most promising industries for low-volume private agriculture in Belarus. The study and promotion of aquaculture has initiated the active creation of many fish farms in recent years. The development of private fish farms is consistent with government goals and allows solving problems of ensuring the country's food security and import substitution of food products.

The mirror carp is one of the most valuable promising objects of fish farming in Belarus. Along with the temperature and feeding regime, protection from diseases and parasites, water quality is the most important factor contributing to the success of carp breeding. On the other hand, breeding fish in ponds, in particular, mirror carp, leads to changes in water quality, resulting in eutrophication and subsequent pollution. In most cases, the ecological status of water bodies is closely related to human activities. The increase in the production of mirror carp in ponds of Belarus will inevitably lead to a deterioration in the ecological status of water bodies and related territories.

This increasing man-made load causes a significant environmental disturbance. Forecasts for the future do not always inspire optimism. This is largely due to the further expansion of the scale of production and the imperfection of the technologies used. Existing development projects for the treatment of industrial waste are often not used for economic reasons. The proposed method of cleaning waste specific production must meet not only the quality requirements, but also be quite simple to use and low-cost.

The theoretical basis of biological rehabilitation is a comprehensive solution to the problems of polluted water bodies. The scheme of biological rehabilitation of water bodies includes actions aimed at minimizing pollutants, improving sanitation, preventing the "blooming" of water by blue-green algae, biological amelioration of higher aquatic vegetation and, finally, fishing.

The most promising method used for the biological rehabilitation of wastewater and polluted water bodies is the green alga from a genus of single-celled green algae belonging to the division Chlorophyta – Chlorella vulgaris strains.

The standardized method of using Chlorella in cleaning does not currently exist. In addition, it is necessary to select specialized strains of Chlorella for various purposes: for the purification of wastewater of various origins, to improve the state of water in drinking water bodies or fish ponds.

In connection with the foregoing, **the goal of our work** is to study the possibility of using the selected Chlorella strain to improve the quality of water in the fish pond.

The development of guidelines for the algolization of fish ponds is envisaged in the case of obtaining a meaningful practical results.

To achieve this goal, we solved a number of **tasks**:

1) Select and characterize fish ponds for research.
2) Assess the state of water in the ponds under study before algolization begins.
3) To select the active strain of chlorella, the most successful in reproduction in water of ponds.
4) To conduct the production of chlorella suspension for algolization of the pond.
5) Assess the dynamics of the variability of water quality indicators after algolization.
6) To assess the effect of algolization on the production of mirror carp.
7) Formulate practical guidelines for algolization of fish ponds.

The **novelty** of our research consists in the selection of the active strain of Chlorella and the selection of unique parameters of algolization of fish ponds in Belarus.

Economic efficiency ща this project is the potential increase in fish productivity after algolization.

The **global benefit** of the project is to minimize the harmful effects of the fish farm on the environment and the biological restoration of the natural balance in the fish-growing region.

1. LITERATURE SEARCH

1.1. Impact of fish cultivation in ponds on the state of water and ecosystems

Aquaculture, the farming of aquatic organisms, has been the agro-industrial activity with the highest growth rate worldwide in the last four decades. From 1970 to 2008 the production of aquaculture organisms grew at a rate of 8.3% per year, compared to less than 2% of fisheries, and 2.9% of livestock (Marcel Martinez-Porchas, Luis R. Martinez-Cordova, 2012). The annual aquaculture production is at present over 60 million tons (including marine plants), with an approximate value of 85 billion dollars (FAO, 2010). The last FAO report revealed that the world population increased by 6.3% from 2004 to 2009, whereas the production of aquatic organisms by aquaculture increased by 31.5% in the same period (FAO, 2010).

Despite the undeniable benefits of aquaculture such as the provision of good quality and accessible food for population and the generation of millions of jobs and billion dollars in budget for the developing countries, the activity is one of the most criticized worldwide, mainly because of the environmental impacts that have been and can be caused.

With or without valid arguments, aquaculture has been accused to be the cause of many environmental, social, economic, and inclusively esthetic problems. Ecosystems are not always as fragile as could be considered, instead, they have remarkable capacity of resiliency, and as long as basic processes are not irretrievably upset, ecosystems will continue to recycle and distribute energy (Frankic A, Hershner C., 2003).

The main negative impacts attributed to the activity are as follows:

1) destruction of natural ecosystems; 2) salinization/acidification of soils; 3) pollution of water for human consumption; 4) eutrophication and nitrification of effluent receiving ecosystems; 5) ecological impacts in natural ecosystems because of the introduction of exotic species; 6) ecological impacts caused by inadequate medication practices; 7) changes on landscape and hydrological patterns; 8) trapping and killing of eggs, larvae, juveniles, and adults of diverse organisms; 9) negative effect on fisheries; 10) in its role as food producer, aquaculture is far from complying an adequate distribution of food. Many of the overwhelming influences are closely linked to the state of ecosystems.

To prevent the mass development of blue-green algae, algolization of the fish pond is carried out by planktonic strains of chlorella. The use of chlorella suspension in fish ponds for algolization significantly strengthens their food supply. Such types of carp as silver carp, grass carp or carp will also help to cope with the excessive reproduction of chlorella in the home pond.

A detailed description of these problems and influences is given in a number of research papers (Stickney R. R., McVey J. P., 2002; Barrington K., Ridler N., Chopin T.,Robinson S., Robinson B., 2010; Smith M.D., Roheim C.A., Crowder L.B., 2010;Roos N., Wahab M. A., Chamnan C., Thilsted S.H., 2007; Frankic A.,Hershner C., 2003; Tidwell J. H., Allan G. L., 2001; Alongi D. M., 2002;Walters B. B., Rönnbäck P., Kovacs J.M., 2008; Naylor R.L., Goldburg R.J.,2000; Focardi S., Corsi I., Franchi E., 2005; Feng Y.Y., Hou L.C., Ping N.X.,Ling T.D., Kyo C.I., 2004; Gyllenhammar A., Håkanson L., 2005; Jackson C., Preston N., Thompson P.J., Burford M., 2003; Deutsch L., Gräslund S., Folke C.,2007; Shelton W.L., Rothbard S., 2006; Krkošek M., Ford J.S., Morton A., Lele S., Myers R.A., Lewis M.A., 2007; Boxall ABA., 2004; Páez-Osuna F., 2001; Boyd C.E., 2003; Read P., Fernandes T. M., 2003).

1.2. Biological characteristics of chlorella

Chlorella is a genus of unicellular plants of green algae. This genus includes about 20 species, and in the Belarus there are about 6 species. The most famous and widespread is Chlorella vulgaris forming huge accumulations in the water of muddy pools, ditches and ponds.

Chlorella cells are single, spherical or ellipsoidal, the diameter does not exceed 15 microns. This immobile microorganism has a thin, smooth cellulose casing. The cell contains one nucleus and only one chloroplast located near the wall. Products such as fats and starch are stored in the cytoplasm (Vladimirova M.G., Semenenko V.E., 1962).

Reproduction. Only asexual reproduction is characteristic, which is carried out by dividing the cell into 4-8-16 equal parts - autospores. These newly formed cells, after the disappearance of the maternal membrane, turn out to be free, quickly increase in size and, after a short period of time, divide again.

Economic value. Chlorella is able to extremely rapidly accumulate biomass during cultivation, so that this microorganism has become the most popular object of cultivation and research. Representatives of this kind of green algae are used for experimental studies in closed ecological life support systems. During the life of chlorella, a large amount of oxygen is released during photosynthesis, which has been used for air regeneration in confined spaces, for example, in space ships, submarines. Research is underway to use chlorella as a likely source of food, but the difficulty is that all the nutrients of the algae are covered with a solid shell that human digestive enzymes cannot destroy. Also of note is the importance of these algae for biological wastewater treatment (Ilmutdin M. Abdulagatov et al, 2018).

Cultivation of Chlorella. In a conventional cumulative culture, as the number of cells increases and conditions change (nutrient medium depletion, self-shadowing of cells, accumulation of in vivo secretions), the rate of division and accumulation of biomass slows down. In such cultures, growth is described, as in bacterial cultures, by an S-shaped curve and can be divided into several stages: 1) lag-phase, 2) logarithmic or exponential growth phase, 3) linear growth phase, 4) plateau (stationary), 5) death phase. The duration of the lag-phase depends on the prehistory of the culture, the period of exponential growth in algal cultures is short, and the faster the increase in cell number occurs, the faster self-shadowing of the culture occurs, the shorter the period of exponential growth. The linear growth stage of the algae culture is the longest (Fankhauser, David B.).

Like all green plants, algae synthesize valuable organic substances from CO2 and water in the process of photosynthesis, such as proteins, fats, carbohydrates, vitamins and other physiologically active compounds.

Within the genus Chlorella there are light-loving and shade-tolerant cultures with a lower or higher chlorophyll content, with a higher or lower chlorophyll unit activity, there are thermophilic, mesophilic, and psychrophilic (well growing at + 5 ... + 10 ° C) strains. Each strain in one way or another has adaptive abilities. But each culture has its own genetically fixed range of requirements for light and temperature (Ilmutdin M. Abdulagatov et al, 2018).

A large number of mesophilic and thermophilic highly productive forms of chlorella were obtained by selection in nature and selection and genetic methods. A comparative study of different strains of algae shows a wide variety of them in relation to temperature, light, growth ability in dense populations. This opens up the possibility of directional selection of forms of algae in relation to specific conditions and goals of their cultivation (Vladimirova M.G., Semenenko V.E., 1962).

The selection and maintenance of promising forms in the collection is an important task.

Cultivation of chlorella requires aqueous solutions containing a source of nitrogen, phosphorus and potassium, calcium and magnesium salts, sulfates and other macronutrients, trace elements, as well as carbon dioxide (Ilmutdin M. Abdulagatov et al, 2018).

1.3. Methods of restoring fish culture ponds

Currently, wastewater treatment is based on bacterial activity. At the same time, a prerequisite is the bubbling of activated sludge with air, from which bacteria absorb oxygen for their vital activity, and carbon dioxide is released to the environment. Viciousness of this purification system consists in the enormous consumption of oxygen for the bacterial processes of destruction of organic substances during the treatment of wastewater. Carbon dioxide - the product of the vital activity of bacteria - is released into the air, i.e. the currently existing sewage treatment plants are oxygen consumers and air pollutants with carbon dioxide. In sanitary terms, bacterial purification does not release sewage from microorganisms that cause diseases in humans and animals.

The biological peculiarity of bacteria lies in the fact that they are highly specialized, that is, there is no one type of bacteria that could clean the entire spectrum of substances that are in the wastewater (Bogdanov, 2001).

Therefore, there is an urgent need to find a different approach to the problem of environmental improvement.

Algae growth is accompanied, above all, by a decrease in the concentration of ammonia nitrogen and mineral phosphorus. Most often, protococcal algae dominate in biological ponds and only occasionally volvox and euglean. The biomass of algae grown on wastewater was not inferior, and often exceeded, obtained on a nutrient medium (Sivko, 1961). The presence of a bactericidal component in the culture fluid of protococcal algae was found. This contributed to the release of wastewater from saprophytic and pathogenic microflora (Levina, 1961).

The process of wastewater treatment from organic and mineral contamination using microalgae is 1.5–2.0 times more intense (Vagisov, 1984). As a result of biological treatment, the hydrobiological regime of wastewater is stabilized. The nitrogenous components and phosphates contained in wastewater are fully utilized by algae (Buriev, 1980).

Bacteriostatic action of Chlorella vulgaris has been identified on the microflora of wastewater from the livestock complex. So, on the third day of algae growth, the number of bacteria growing on MPA (meat-peptone agar) decreased by 1000, and of E. coli by 100 (Bilmes, 1984).

Ecologically, this is justified by the fact that algae use mineral (biogenic) substances, carbon dioxide for their vital activity, and in the process of vital activity they release oxygen into the environment. Evolutionary algae are at a higher stage of development, for example, compared to bacteria, and therefore many species are universal consumers of organic and mineral substances.

1.4. The mechanism of action of chlorella suspension

In the case of algolization of water bodies, the question of the mechanism of chlorella's effect on blue-green algae is of particular interest. In laboratory experiments, it was shown that in the presence of chlorella, blue-green algae cells that cause water to bloom are lysed. At the same time, other types of blue-green algae Chlorella does not have any lytic or inhibitory effects, and some of them, for example, Oscillatoria sancta, develop more intensively in the presence of Chlorella vulgaris. Consequently, chlorella has a selective effect and, moreover, only on those types of algae, which traditionally cause blooming of water.

It should be noted that chlorella releases to the environment more than 310 chemical compounds (Stanchev, 1980), among which are biologically active polysaccharides, for example, HlA-A (Melnikov, Manannikova, 1991), various organic acids (parinary, arachidonic, linolenic, etc.) with a strong antimicrobial effect, amines (26 items), the increased concentration of which in the medium may cause inhibition of growth of some algae and stimulate the development of others (Sakevich, 1985). The composition and amount of intravital secretions are very diverse (Fogg et al., 1965; Moore, Tischer, 1965). Perhaps, one should not exclude the fact that chlorella can react to exometabolites, which release blue-green algae “bloom” of water with the production of abscisic acid (opened in 1963), belonging to the strongest natural growth inhibitors (Sirenko, Kozitskaya, 1988).

That is, algae, including chlorella, have a large composition of chemical compounds that can one way or another regulate the relationship between species of the phytoplankton community. Another feature of chlorella is that algolization of a reservoir during the flowering period does not have a positive effect on it. Therefore, the settlement of the reservoir with chlorella should be carried out in a period when the same starting conditions can be created for all types of algae, i.e. in autumn or spring. As a result, the predominant development is received by a species endowed with greater biological activity.

2. MATERIALS AND METHODS

2.1. Periods of study

The primary study of fish ponds began in 2016 with a survey of ponds, studying the flora and fauna of ponds, taking water samples and assessing its quality with portable minilaboratories and using accurate methods. In 2017, Studies on water analysis were continued to assess the dynamics of changes in its composition before and after algolization. At the same time, the effect of chlorella on the microbiological composition of water was studied. The amount of chlorella in the control and test ponds was studied.

In 2017, the chemical and microbiological components of water were studied and a decision was made on algolization. We also studied local strains of chlorella and isolated the most optimal strains. We also evaluated the ability of chlorella strains to be cultivated under laboratory conditions in order to select the optimal conditions for increasing biomass for subsequent algolization.

In 2018, algolization was performed. Studies on water analysis were continued to assess the dynamics of changes in its composition. At the same time, the effect of chlorella on the microbiological composition of water was studied. The effect of algolization on the mirror carp productivity was also studied.

2.2. Characteristics of the studied reservoirs

Koritsa village is located in the Dyatlovsky district. The river Lopushanka "Akulovka" flows through its territory, the length of which is 6 km. It forms a dam with an old lake, an area of 2.53 hectares. On the territory of numerous springs. In the forest dominated by alder, pine, shrubs. On the shore of the lake is dominated by plants such as horsetail, sedge, tart buttercup, peat moss, reed. The surveyed reservoir does not experience strong anthropogenic pressures in the area of research. The natural reservoir was rented by our families.

Fig. 1. Pond 1 in Koritsa village.

The area we rent a nursery pond was 2.53 hectares. This pond is located on low-lying areas with high soil water. The depth of the pond varies from 20cm to 3 meters. The pond descends and is fully fished. The constructive device of hydro constructions ensures the maintenance of the design level of water, as well as the impossibility of exiting fish from ponds and entering foreign fish in them. We prepared the pond in spring 2018, where we made an embankment in order to separate the 2 small water basins (1 – control with preserving the usual technology of growing fish; 2 – experimental for algolization) from the main pond, with an area of 0.5 hectares. In the ponds dead vegetation was removed, cleaned of debris.

For the growth of green algae in the pond it is necessary in advance (10-15 days) to make in the reservoir organic and mineral fertilizers (the dosage of which depends on the state of the pond). This is important, as there is competition in the diet between the larvae of fish and the larvae of insects, tadpoles, and small crustaceans. For example, for average indicators, the dosage per 1 hectare of water is as follows: ammonium nitrate 5-10 kg (1 time in 10 days), 1 kg superphosphate (1 time in 10 days) - ammonium nitrate and superphosphate are previously diluted with water. Organic fertilizers are applied once a year: rotted cow dung is 5 tons per hectare. - manure is decomposed along the entire coastline of the pond and is applied in even parts throughout the summer season with mineral fertilizers. Chalk or hydrated lime 100-200 kg. on 1 hectare of the reservoir - make one time. In the spring of 2018, the ponds were flooded (Figure 2).

Fig. 2. Pond 2 (part of pond in Koritsa village) before water flooded.

2.3. Sampling of water and fish

Samples for hydrobiological and microbiological indicators and for analysis were taken during the planned period, i.e. in April - October 2018 in ponds №1 and №2. It is necessary to monitor and control some key parameters of water quality: the level of dissolved oxygen, pH, temperature, total nitrogen content, and others.

Sampling of water (average samples) was carried out from a depth of 0-30 cm in accordance with the methodology (GOST 17.1.5.05-85) in 5 different points.

Control fishing was carried out with a fishnet. In each case, at least 40 specimens of mirror carp were evaluated.

2.4. Estimation of water parameters

To estimate most of the pond water parameters, we used an I-160 ionomer with a set of electrodes (Figure 3), which is designed to determine in aqueous solutions the activity of hydrogen ions (pH), redox potential (Eh), activity and concentration of ions: H +, Li +, Na +, K +, NH4 +, Ag +, X +, NO3-, ClO4-, F-, Cl-, Br-, I-, CN-, SCN-, Ca ++, Ba ++, Mg ++, (Ca + Mg) ++, Pb ++, Cd ++, Cu ++, Hg ++, X ++, CO3--, S - and others. Calibration graphs based on solutions with a known concentration were used to determine the concentration of the investigated ions in the water samples of the control and experimental ponds.

Fig. 3. Conducting measurements of the concentration of water components using I-160 ionomer.

We also used in the work Photometer photoelectric (photoelectric colorimeter) KFK-3 to estimate the concentrations of substances using the photocolorimetric method (Figure 4). In this case, calibration graphs were also used to estimate the concentration of substances.

Fig. 4. Work with Photometer photoelectric (photoelectric colorimeter) KFK-3.

We also used the compact field minilabs of the Visocolor ECO series for rapid assessment of water conditions.

<u>Water smell</u>

Evaluation of the smell of water was carried out according to the method (Determination of Odour Concentration …, 2001) using a qualitative and quantitative scale (Pedro Hernandes, 2018):

Ea	Earthy/Musty/Mouldy
Cl	Chlorinous
Gr	Grassy/Hay/Straw/Wood
Ma	Marshy/Swampy/Septic/Sulphurous
Fr	Fragrant (vegetable or flowery)
Fi	Fishy
Me	Medicinal/Phenolic/Alcoholic
Ch	Chemical/Hydrocarbon/Miscellaneous

Description	Scale
Odour Free	-
Threshold	t
Weak	2
-	4
Moderate	6
-	8
Strong	10

<u>Water transparency and water colour</u>

We determined these parameters in the field based on the methodology developed by Pavel Schlyaga and presented in 2018 at the SJWP-2018 (Figure 5).

Fig. 5. A device for assessing turbidity (transparency) and water color. The camera Canon and the Photoshop program were also used to evaluate the color of the water.

2.5. Cultivation of Chlorella

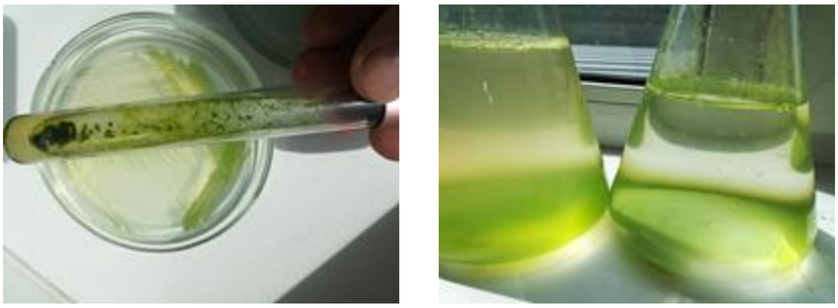
Chlorella culture medium for use in laboratory conditions was prepared with the following composition (for 1 liter of tap water):

Ammonium nitrate, 10 % solution 0.2-0.4 ml; Potassium monophosphate, 10% solution 0.3-0.5 ml; Sodium phosphate disubstituted, 10 % solution 0.05-0.1 ml; Ferric chloride, 1% solution 0,15ml; Cobalt nitrate, 0.1% solution 0.1 ml; Copper sulfate, 0.1% solution 0.1 ml. The selection of strains was carried out on the agar medium based on water of pond.

The selection of fast-growing chlorella colonies was carried out on Petri dishes in a sterile agarized medium based on water from the pond, according to the estimated colony size, 7 days after seeding.

The study of the growth rate of chlorella was performed in a liquid medium based on sterile water from a pond by microscopy.

The ability of chlorella to grow in a liquid medium without sedimentation and fouling was determined by cultivation in a liquid medium based on sterile water from a pond in flasks.



A B

Fig. 6. Cultivation and evaluation of chlorella in the laboratory (A – cultivation in Petri dishes and test tubes; B - evaluation of the growth of strains in flasks).

2.6. Algolization of pond

For inoculation, the best Chlorella vulgaris strain among those selected by us was used. For further dilution, a capacity in the volume of 40 liters was taken, and a cylinder with carbon dioxide was attached to it. The installation was assembled in the first decade of May. The daily air temperature was about 25-29 degrees. The tank was filled with fresh water and the nutrients listed above (Figure 7). A 200 ml culture of chlorella was added to the tank. The rapid growth of chlorella under the conditions created allowed to reach the required number of cells within three days (Figure 8).

After the slurry density chlorella cells reach 50-60 million/ml, it was in an amount of 40 liters portion added to the middle of the pond 2.

Fig. 7. A - Preparation of samples of salts for the cultivation of Chlorella; B - The introduction of the starter chlorella in the nutrient solution

A B C

Figure . 8. Changes in the visual density of chlorella cells during cultivation (A– first day, B – second day, C – third day after inoculation).

Cell density was determined by counting the cells in the field of view. After carrying out practical work for several months, we learned to determine with sufficient accuracy the density of the culture of chlorella even by eye.

Fig. 9. Selection of initial culture for subsequent Chlorella breeding.

2.7. Pond fish stocking

To obtain carp larvae, we independently isolated the sex products of males and females of mirror carp (Figure 10), fertilized eggs (Figure 11), incubated in a device made with our own hands (Figure 12), grown (Figure 13) and released into a pond.

Three days old carp larvae (Figure 13) were introduced into pond 1 and pond 2 on May 10 in the amount of 5,000 individuals into each pond. The second colonization of the larvae was on May 17 in the amount of 5,000 and 50,000 pieces, the third settlement of the planting material was carried out by a 14-day-old growth larva on May 27. Observed the development and growth of fish, conducting intermediate fishing of the bred larvae after 14 days for each stage is May 22, May 30, June 14 2018.

Fig. 10. Mature specimens of carp.

A B

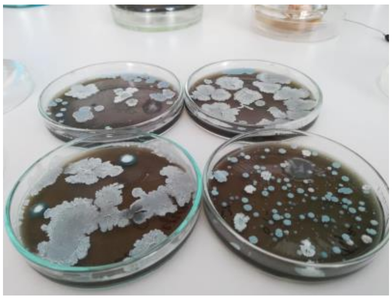
Fig. 11. A - Fertilizing of eggs, B - Apparatus for incubating carp caviar.

Fig. 12. Home pool for growing carp larvae.

Fig. 13. Larva of mirror carp.

2.8. Evaluation of bacterial contamination of pond water

Assessment of bacterial contamination of pond water is important for assessing the general state of water, its saturation with organic and other substances. Too much bacterial growth can reduce the content of dissolved oxygen, affect negatively the general condition of the pond ecosystem and the cultivation of fish. Evaluation of bacterial contamination was carried out using standard nutrient medium based on peptone and glucose with the addition of 1.5% agar; a different dilution of water in an amount of 0.1 ml was sown on Petri dishes with a spatula. Bacteria were cultivated at 28 degrees Celsius. Counting colonies were conducted visually.



A B

Fig. 14. Microbiological studies (A – bacterial colonies; B - Counting the number of colonies / bacteria in different dilutions of water samples from the pond)

2.8. Microscopy.

Microscopic studies were performed using light-field microscopy. Microscopic examination was carried out at a magnification of 400-1000 times. A Levenhuk 625 microscope was used (Figure 15). For cell counting, Goryaev’s Camera (cytometer) and spectrophotometer were used. Microscopic examination was necessary for counting chlorella cells in culture, as well as counting algae cells in pond water samples.

A B

Fig. 15. A – General view of a Levenhuk 625 microscope. B – Sample preparation and microscopic examination.

3. RESULTS AND DISCUSSION

3.1. Isolation of the active strain of chlorella

As shown in the literature review, for each task (production of protein or oil, wastewater treatment of various origins, algolization, and so on), a specific chlorella strain is required that will be best suited to solve the actual problem.

For algolization, the growth rate under the existing conditions that cannot be controlled, low sedimentation to the bottom, resistance to limiting factors and the ability to effectively solve the problem (excess nitrogen, phosphorus, blue-green algae, etc.) are important.

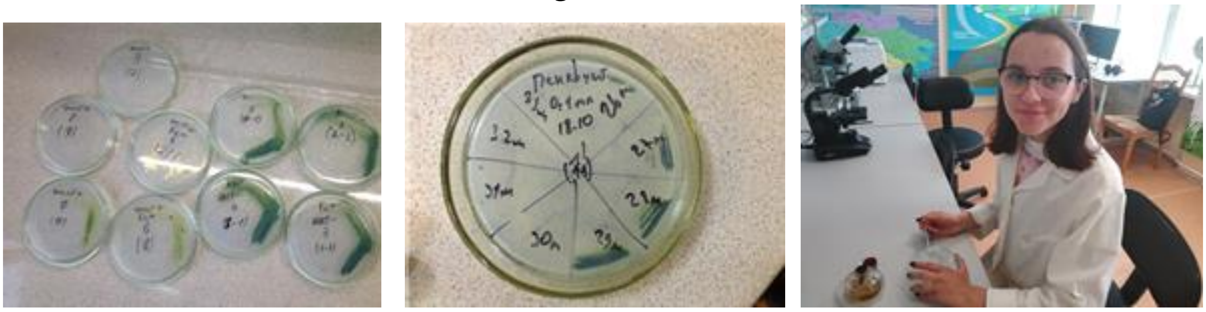
Based on the task, we have used pond water as a medium for selecting the chlorella strain. Selection was carried out by us by selecting isolated colonies that are progeny of single cells (Figure 16).

Under these conditions, the selection was carried out on the growth rate (Figure 17) and the ability to stay suspended in water for as long as possible (Figure 18).

As a result, we received our own strain lab-3, which satisfied the specified conditions.

A B C

Fig. 16.A – Biomass of Chlorella cells. B – Isolated colonies of Chlorella vulgaris vary in size; C - Observing Chlorella colonies.



A B C

Fig. 17. A - Check for homogeneity of isolated strains of Chlorella vulgaris. B - Preserving and maintaining chlorella strains in the laboratory; C - Sowing of dilutions of chlorella on Petri dishes with a spatula.

Fig. 18. Evaluation of cell sedimentation rate in the laboratory.

3.2. Assessment of the dynamics of changes in water quality indicators after algolization

<u>Content of nitrates (mg/l)</u>

No. of pond	Dates (2018) of water sampling and analysis						
	April	**May**	**June**	**July**	**August**	**September**	**October**
1 (control)	1,24	1,32	0,75	0,7	0,68	0,42	0,31
2	1,21	1,05	0,62	0,54	0,58	0,39	0,29

Algolization has a significant effect on nitrate content. The nitrate content becomes lower and more stable. A higher concentration in spring is due to the washing away of organic matter and the decomposition of vegetation. (Filling water was carried out from a natural reservoir - the lake.)

Content of nitrites (mg/l)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	0,01	0,02	0,028	0,026	0,03	0,02	0,00
2	0,01	0,015	0,020	0,012	0,01	0,00	0,00

Algolization has a significant effect on nitrite content. The nitrate content becomes lower and more stable. This is probably due to a change in the nitrogen cycle in the reservoir after algolization.

Content of ammonium (mg/l)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	0,212	0,225	0,162	0,145	0,177	0,154	0,092
2	0,220	0,232	0,130	0,135	0,142	0,138	0,084

Algolization has a significant effect on the ammonium content. The ammonium content becomes lower and more stable. Higher concentration in spring due to flushing of organic matter.

Content of phosphates (mg/l)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	0,420	0,520	0,260	0,380	0,260	0,450	0,320
2	0,480	0,560	0,120	0,180	0,130	0,340	0,280

Phosphates are an important nutrient element and changes in dinacmics and phosphate content in ode may be due to the vital activity and development of green algae after algolization.

Content of iron (mg/l)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	0,720	1,300	0,480	0,480	0,120	0,230	0,380
2	0,680	1,450	0,320	0,355	0,100	0,100	0,210

Algolization affected the total iron content in water. This may be due to the binding, precipitation and consumption of iron by chlorella and the creation of favorable conditions for the reproduction of green microalgae.

Content of oxygen (mg/l)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	4,9	4,6	4,0	3,8	3,7	4,9	5,2
2	4,9	4,8	6,2	6,8	6,3	5,2	5,4

The change in oxygen content is caused by the level of decomposition of organic matter, photosynthesis, temperature and other factors. Algolization has had the most significant positive effect on this indicator.

Water acidity (pH)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	7,52	7,38	8,22	8,58	8,92	7,85	7,28
2	7,58	7,42	8,52	8,91	9,12	7,92	7,15

The water in the pond after algolization in mid-summer was characterized by greater alkalinity, which may be due to more intensive photosynthesis.

Water smell (point, description (predominant is the first))

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	t, Gr	2, Gr	5,	7, Fi, Ch, Ma	9, Ma,Fi	3, Fi	t, Fi, Gr
2	t, Gr	2, Gr	5, Gr	6, Gr, Ch	7, Gr, Fi	3, Gr	t, Gr

Algolization has a significant effect on the smell of water. There is no sulfur smell and the smell of a septic tank during algolization.

Water transparency (thickness of a distinguishable line)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	2	4	4	5	7	3	1
2	2	4	5	6	6	3	1

Algolization affects the turbidity of water, which increases with algolization, which is associated with the best reproduction of algae.

Water colour (R/G/B and approximate color illustration)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	Sept.	October
1 (control)	246/246/242	230/251/100	232/248/195	209/254/186	163/253/167	189/233/191	222/231/212
2	250/248/241	230/256/102	213/253/150	215/254/109	210/255/115	196/251/175	231/236/214

Algolization has a significant effect on the color of water, which changes in the direction of enhancing the green color during algolization, which is associated with the best reproduction of green algae.

Water surface temperature (°C)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	16	22	24	23	25	19	12
2	17	23	24	22	26	19	12

Thus, algolization does not affect the water temperature.

Level of water bloom

We carry out a quantitative assessment of the presence of bluegreen algae, but also phytoplankton was cultivated in flasks — control from pond 1 and experimental from pond 2. Sampling was carried out before algolization and then monthly.

In all flasks of the control variant, we observed the presence of blue-green fouling on the 12th day. In the experimental flasks, blue-green fouling was observed only in samples from April (before algolization) and in early May. In all other periods, the presence of blue-green fouling was no longer observed. This demonstrates a significant effect of algolization on the development of blue-green algae.

Bacterial contamination (general) of water (CFUx1000/ml)

No. of pond	Dates (2018) of water sampling and analysis						
	April	May	June	July	August	September	October
1 (control)	312,48	962,74	542,12	555,25	769,36	338,71	120,20
2	362,15	413,89	420,56	306,34	309,28	220,25	109,22

As can be seen from the above data, algolization had a significant impact on the bacterial contamination of the pond and this effect is positive.

Considering that the saprophytes studied by us are indicators of organic pollution of the reservoir, it can be stated that pond 1 contains an excess of nutrients, while chlorella reduced the availability of mineral nutrients to bacteria and contributed to the restoration of normal state of the pond.

The change in the amount of microalgae (cells/ml), (01 August – usually the time of active blooming of water).

No. of pond	Number of cells (cells/ml)				
	General	Green	Diatoms	Bluegreen	Others
1 (control)	97 200	95 200	672	868	460
2	106 800	105 821	438	6	535

Thus, algolization has a significant impact on the development of microalgae.

3.3. Evaluation of the effect of algolization on the productivity of mirror carp

The total duration of cultivation of larvae of mirror carp fish lasted 4 months. Observing the development and growth of fish, having carried out an intermediate harvest of an adolescent larva after 14 days for each stage is May 22, June 6, June 20. The larvae were recorded height and weight: 12 mm., 20 mg. Samples of biomaterial from pond 1 were not taken into account due to difficulties in catching the larvae. After 30 days, a second intermediate harvest in the pond 2 was carried out: as a result, the growth of larvae averaged 3 cm, and the weight was 6 grams. The third intermediate fishing was also carried out a month later on July 20. Growth fry ranged from 5-7 cm., Weight from 15-25 grams.

When conducting a control fish catch on September 25 in the pond 1, the average weight of the fingerlings was 25-35 grams, the body length varied on average 10-12 cm. And in the pond 2 the weight of the fingerlings of the first sample ranged from 60 to 120 grams, the length of the body was 15 -17 cm. Second samples from 50 -70 grams, third samples 30-50 grams. The body length of the fish of the second sample is 12-14 cm, the third is 10-12 cm.

To assess the statistically significant differences, we used Student's criterion, which is designed to compare indicators and shows not only the severity, but also the direction of change. Calculations according to the criterion we made online. It was found that there is a statistically significant difference in the growth of fish in the pond number 2 ($p < 0.05$) from fish in the pond number 1.

Weighing fish, depending on its size, was carried out on a technical scale. When weighing, the fish were pre-dried with filter paper or gauze. The growth rate is measured in absolute or relative terms. The growth rate in terms of absolute growth can be expressed by the formula $A = V2-V1 / T2-T1$, where A is the absolute increase in fish; V2 is the size, or weight, of fish at the end of a period; V1 - the size, or weight, of fish at the beginning of the period; t2-t1 is the period time.

To judge the relative growth rate, the relative growth, or relative growth rate, is calculated. The expression of the growth rate, not in absolute, but in relative terms, makes it possible to judge the intensity of the growth process. The end result is usually expressed as a percentage. The calculation is carried out according to the following formula: $R = V2-V1 / 0.5 * (V2 + V1) * 100$.

Calculating the absolute and relative growth rate for mirror carp, we obtained the following results: the average monthly increase of mirror carp is 70% in pond 2, which is 50% higher than the growth rates of these fish in pond 1 (control).

Thus, the fish productivity of pond 2 is 233% higher than the fish productivity of pond 1. Is this achieved thanks to? Obviously, the activity of chlorella in water changes the phytoplankton species composition of a water body towards the advanced development of green algae, which displace blue-green algae that adversely affect water quality, as well as the rapid development of zooplankton in the pond 2, which made it possible to obtain a standard sample of carp yearlings ahead of schedule Growing the larva of three ages in the test ponds, fingerlings of the first planting material showed an optimal result compared to the second and third yearling juveniles, which suggests that the optimal time for fish planting material was earlier introduction into the reservoir. In the future, we are planning an experiment on algolization of ponds using planting material from other breeds of pond fish such as silver carp motley, grass carp, European catfish, etc.

CONCLUSIONS

1. To prevent the mass development of blue-green algae and biological recovery of pond water, it is possible to carry out algolization of the fish pond with planktonic strains of chlorella (Chlorella vulgaris strain lab-3).

2. In a mini installation at home, you can successfully cultivate chlorella, which allows you to get about 40 liters of live algae biomass from 200 ml of suspension for three days.

3. With introduction of chlorella into the fish pond 2 with an area of 0.50 ha at spring 2018, the number of aquatic organism increases, the hydrochemical, especially oxygen regime of reservoirs improves, which increases the productivity of fish ponds by 2.5 times compared to the average standards for hinged carp mirror.

4. Growing three-age mirror carp larvae in the test ponds, fingerlings of the first planting material showed optimal results compared to the second and third yearling fingerlings, which suggests that the optimum time for fish planting material was earlier introduction into the reservoir.

This method of algolization of ponds is suitable for reservoirs with a high density of fish landing. The use of this technique is suitable for small farms in the Republic of Belarus.

LITERATURE USED AND CITRD

1. Alongi DM. Present state and future of the world's mangrove forests. Environmental Conservation. 2002;29(3):331–349.
2. Barrington K, Ridler N, Chopin T, Robinson S, Robinson B. Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquaculture International. 2010;18(2):201–211.
3. Boxall ABA. The environmental side effects of medication. EMBO Reports. 2004;5(12):1110–1116.
4. Boyd CE. Guidelines for aquaculture effluent management at the farm-level. Aquaculture. 2003;226(1–4):101–112.
5. Determination of Odour Concentration in Water by Danish Cooperation for Environment and Development, 2001 (from: http://www.ecoideal.com.my/ danidaurban/uems/download/REQ%2020%20Determination%20of%20Odour%20Concentration%20in%20Water.pdf).
6. Deutsch L, Gräslund S, Folke C, et al. Feeding aquaculture growth through globalization: exploitation of marine ecosystems for fishmeal. Global Environmental Change. 2007;17(2):238–249.
7. Fankhauser, David B. Bacterial Growth Curve / University of Cincinnati Clermont College, 2016.
8. FAO. World Review of Fisheries and Aquaculture. 2010.
9. Feng YY, Hou LC, Ping NX, Ling TD, Kyo CI. Development of mariculture and its impacts in Chinese coastal waters. Reviews in Fish Biology and Fisheries. 2004;14(1):1–10.
10. Focardi S, Corsi I, Franchi E. Safety issues and sustainable development of European aquaculture: new tools for environmentally sound aquaculture. Aquaculture International. 2005;13(1-2):3–17.
11. Frankic A, Hershner C. Sustainable aquaculture: developing the promise of aquaculture. Aquaculture International. 2003;11(6):517–530.
12. Frankic A, Hershner C. Sustainable aquaculture: developing the promise of aquaculture. Aquaculture International. 2003;11(6):517–530.
13. GOST 17.1.5.05-85 Nature Protection (SSOP). Hydrosphere. General requirements for sampling surface and sea waters, ice and precipitation. (in Russian).
14. Gyllenhammar A, Håkanson L. Environmental consequence analyses of fish farm emissions related to different scales and exemplified by data from the Baltic—a review. Marine Environmental Research. 2005;60(2):211–243. [PubMed]
15. Ilmutdin M. Abdulagatov, Alibek B. Alkhasov, Gasan D. Dogeev, Nariman R. Tumalaev, Rasul M. Aliev, 1Gasan B. Badavov, Aslan M. Aliev, Asiyat S. Salikhova Technological application of microalgae in power industry and environmental protection // South of Russia: ecology, development. Vol.13 no.1 2018. P. 166-183. (in Russian).
16. Jackson C, Preston N, Thompson PJ, Burford M. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. Aquaculture. 2003;218(1–4):397–411.
17. Krkošek M, Ford JS, Morton A, Lele S, Myers RA, Lewis MA. Declining wild salmon populations in relation to parasites from farm salmon. Science. 2007;318(5857):1772–1775.
18. Marcel Martinez-Porchas, Luis R. Martinez-Cordova. World Aquaculture: Environmental Impacts and Troubleshooting Alternatives // ScientificWorldJournal. 2012. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3353277/)
19. Naylor RL, Goldburg RJ, Primavera JH, et al. Effect of aquaculture on world fish supplies. Nature. 2000;405(6790):1017–1024.
20. Páez-Osuna F. The environmental impact of shrimp aquaculture: a global perspective. Environmental Pollution. 2001;112(2):229–231.

21. Pavel Schlyaga. The definition of colour, colouring and turbidity of the water // The Stockholm Junior Water Prize. 2018 Finalists. P. 5.

22. Pedro Hernandes. Odor Testing for Drinking Water, 2018 (https://alsglobal.blog/en/odor-test-to-water-potable/).

23. Read P, Fernandes T. Management of environmental impacts of marine aquaculture in Europe. Aquaculture. 2003;226(1–4):139–163.

24. Roos N, Wahab MA, Chamnan C, Thilsted SH. The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. Journal of Nutrition. 2007;137(4):1106–1109. [PubMed]

25. Shelton WL, Rothbard S. Exotic species in global aquaculture—a review. Israeli Journal of Aquaculture-Bamidgeh. 2006;58(1):3–28.

26. Smith MD, Roheim CA, Crowder LB, et al. Sustainability and global seafood. Science. 2010;327(5967):784–786. [PubMed]

27. Stickney RR, McVey JP. Responsible Marine Aquaculture. New York, NY, USA: World Aquaculture Society; 2002.

28. Tidwell JH, Allan GL. Fish as food: aquaculture's contribution. Ecological and economic impacts and contributions of fish farming and capture fisheries. EMBO Reports. 2001;2(11):958–963.

29. Vladimirova M.G., Semenenko V.E. Intensivnaya cultura odnokletochnykh vodorosley [Intensive Culture of Unicellular Algae]. Moscow, AN SSSR Publ., 1962, 59 p.

30. Walters BB, Rönnbäck P, Kovacs JM, et al. Ethnobiology, socio-economics and management of mangrove forests: a review. Aquatic Botany. 2008;89(2):220–236.