Int.J.Curr.Microbiol.App.Sci (2023) *12*(07): 1-15

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 7 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1207.001

Problems and Prospects of the Use of Macrophytes and Microalgae in Sectors of the Economy (Scientific Analysis on the Example of the Republic of Uzbekistan and Countries of the World)

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Keywords

Microalgae, Ankistrodesmus, Botryococcus, Scenedesmus, Chlorella, Chlorococcum

Article Info

Received: 02 June 2023
Accepted: 05 July 2023
Available Online: 10 July 2023

ABSTRACT

This article provides information on the problems and prospects of production processes based on microalgae and macrophytes in the conditions of Uzbekistan. The research works conducted in Uzbekistan on the use of microalgae in fisheries, the importance of microalgae in the cultivation of zooplankton, wastewater treatment based on higher algae, that is, macrophytes, and the use of Azolla and small ryaska in the production of feed and supplements are described. Based on research, the dependence of protein and fat storage, which determines the nutritional value of microalgae, was studied on the generations of microalgae, and it was noted that the accumulation of protein and fat by microalgae differed sharply from each other in the Chu-13 feed medium. According to the obtained results, it is recommended to use *Botryococcus* and *Chlorococcum* genera strains in obtaining feed with complete nutritional content for the aquaculture industry. Also, in this research work, microalgae (*Botryococcus*, *Chlorococcum*), which are one of the essential links in the food chain of zooplanktons used as a live feed, are recommended as a natural food source in the artificial cultivation of zooplanktons. New nutrient media was developed based on mixtures of macrophytes Lemna minor L. flour (protein - 16.10%), wheat bran (protein - 14.2%), and Azolla carolina L. (protein - 27.6%). Also, as a result of the implementation of the technology of industrial cultivation of Lemna minor macrophyte, the composition of expensive feed products such as wheat flour, wheat bran, corn flour, and soybean meal, is used for the cultivation of nutritious insects and macrophytes, which are non-traditional sources of food, was improved based on azolla and small ryaska. It is also recommended for the biological treatment of chromium-contaminated wastewater based on high-algae Azolla. In particular, it was noted that the tolerance of higher algae to chromium is different. In studies of the tolerance of different types of higher algae to chromium, it was noted that Azolla is somewhat resistant to other higher algae in the experiment.

Introduction

The role of microalgae in the economy

Microalgae are fast-growing microorganisms, characterized by excellent biochemical properties. Therefore, microalgae were considered an important object in preparing extremely important biopreparations and raw materials for various sectors of the economy. Including food additives, lipids, enzymes, protein, starch, phycocolloids, polymers, toxins, pigments, vitamins, antioxidants, stable isotope biochemicals, environmentally friendly energy products, biofuel, bioethanol, etc. (Chen *et al.,* 2014a; Bleakley and Hayes, 2017; Moreno-Garcia *et al.,* 2017; Pemmaraju *et al.,* 2018).

When classifying the branches of application microalgae in practice by the countries of the world community, microalgae are used in the fields of human food, biologically active substances in nutraceuticals, feed, and additives for animals and aquatic animals, cosmetics, and skin processing products, as well as anti-colds for the biomedical and pharmaceutical industries. Besides, it is widely used as a raw material for the synthesis of anti-thrombogenic, anti-atherogenic products (Khujamshukurov, 2011a; Suganya *et al.,* 2016; Wells *et al.,* 2017; Sassi *et al.,* 2019; Ortiz-Moreno *et al.,* 2020).

In the world, there are serious problems related to feed and feed additives in providing people with nutritious and rich in all necessary ingredients, environmentally friendly food products, including fish and fish products. Also, the role of natural sources of feed (zooplankton organisms, microalgae, macrophytes, insect organisms, etc.) is special for the continuous supply of feed products to the fishing industry, and it is important with the possibility of covering 30-40% of the total feed consumption.

Traditional and non-traditional feed products are being used for the production of feed products to continuously supply the world's fishing industry with feed products. In particular, if agricultural and food sources such as wheat grains, corn, and millet are used as traditional feed products, extensive scientific research is being conducted on the use of zooplankton organisms, microalgae, macrophytes, and nutritious insects as non-traditional feed products.

In this regard, special attention is also paid to the use of microalgae and macrophytic organisms for the cultivation of zooplankton, as feed products for the fishery network based on nutritious insects, as well as the use of biomass of microalgae and macrophytic organisms.

In addition, the use of macrophytes and microalgae in areas such as the removal of heavy metal ions from wastewater, monitoring of toxicants in the environment, bioassays, and organic bioremediation is expanding day by day (Iyovo *et al.,* 2010; Subashchandrabose *et al.,* 2011; Rizwan *et al.,* 2018). It is also noted that microalgae have great potential as an inexpensive and environmentally safe expression system (Ortiz-Moreno *et al.,* 2020). In particular, research is being conducted on obtaining composite materials based on *Chlorella* vulgaris as a filler for various polymers (Wijffels, 2013). Some commercially used microalgae include Arthrospira (Spirulina), Chaetoceros, *Chlorella*, Dunaliella, and Isochrysis (Odjaddare *et al.,* 2017; Rizwan *et al.,* 2018).

The role of macrophytes in the economy

Macrophytes belonging to the family Lemnaceae are distinguished by their importance in various areas of the economy. In particular, it is an effective source in the production of food and feed products, and is widely used to clean wastewater and chemically contaminated water bodies from heavy metals and pesticides (Mkandawire *et al.,* 2007; Radić *et al.,* 2011).

Industrial production of products based on representatives of the Lemnaceae family is widely established, and one of the main ones is the production of flour based on ryaska, ingredients rich in fatty acids, feed additives for poultry for various purposes, and protein feed for fish (Haustein *et al.*, 1994; Bairagi *et al.*, 2002). It is noted that flour prepared based on Lemnamacrophytes contains 35-45% crude protein and 7-10% fiber (Olorunfemi *et al.*, 2006; Hasan and Chakrabarty, 2009; Rojas *et al.*, 2014). Parabel Inc. (Melbourne, FL) company has put into practice the production of LPC (Lemna protein concentrate) based on the new technology of extracting crude protein and non-exchangeable amino acids based on Lemna species, which is characterized by about 68% crude protein content in LPC (Rojas *et al.*, 2014).

In scientific sources, Lemna spp. based on which annual production indicators are recorded. In particular, 60-145 t/ha/year in Thailand-Vietnam regions (Landolt and Kandeler, 1987), 36-51 t/ha/year in Israel (Leng *et al.*, 1995), 7-8 t/ha/year in Russia (FAO, 2001), 7-15 t/ha/year in Uzbekistan (FAO, 2001), 22-34 t/ha/year in Germany (Mkandawire and Dudel, 2005), 30-70 t/ha/year in India (Leng *et al.*, 1995), 30 t/ha/year in Egypt (Landolt and Kandeler, 1987) and 57-185 t/ha/year in various regions of the USA (FAO, 2001) have been recorded.

Now it can be seen that these indicators have increased several hundred times. In particular, in the conditions of Uzbekistan, the weather is comfortable, sunny days are almost 308 days, and there is an opportunity to continue growing macrophytes from March to November. Also, based on our scientific research, it has been proven that it is possible to obtain wet biomass in the amount of 154 t/ha/year or 27.34 tons of dry mass in the conditions of Uzbekistan (Khujamshukurov, 2011b).

It is known that the output of dry biomass from the composition of any object, i.e. from wet biomass, is very important in production conditions. From various scientific sources, their dry mass formation in Lemna-based products is 3.5% (Landesman, 2000), 4.9% (Goopy *et al.*, 2003), 5.1% (Rusoff *et al.*, 1980) cited (FAO, 2001). This is the reason for the calculation of dry mass from their wet biomass, different indicators of the amount of protein in the products based on them.

Commercial production of Lemnaceae has been widely launched based on industrial production of products, one of the mains of which is leading the production of ryaska-based flour, ingredients rich in fatty acids, feed additives for poultry for various purposes, protein feed for fish (Haustein *et al.*, 1994; Bairagi *et al.*, 2002). It is noted that flour prepared based on Lemnamacrophytes contains 35-45% crude protein and 7-10% fiber (Olorunfemi *et al.*, 2006; Hasan and Chakrabarty, 2009; Rojas *et al.*, 2014). Parabel Inc. (Melbourne, FL) company has put into practice the production of LPC (Lemna protein concentrate) based on the new technology of extracting crude protein and non-exchangeable amino acids based on Lemna species, which is characterized by about 68% crude protein content in LPC (Rojas *et al.*, 2014).

Large-scale scientific research on microalgae is also being conducted in Uzbekistan. In particular, the objects of microalgae listed in Table 1 are widely used in many sectors of the economy, including in pharmaceuticals for the production of biologically active substances, vitamins, emollient and tightening gels for wound healing, antioxidant preparations, protein-vitamin-rich bioactive additives in the food industry, high lipid preservatives, recommended as an algological source for the use of children's food products as protein-vitamin-lipid enrichment agents.

Also, in recent years, it has been widely used as the main source of bioethanol and biodiesel, one of the most urgent issues (Khujamshukurov *et al.*, 2016). In addition, it is widely used for the production of nutritious feed products for the poultry and fishery industry and as live feed (Haydarov *et al.*, 2020a, b).

The environmental management with Azolla

When quality water is scarce, low-quality water or even wastewater must be considered for agricultural use. The use of agricultural wastewater can play an important role when disposal is planned in arid and semi-arid regions. In terms of irrigation, the use of low-quality wastewater requires more complex management methods and stricter control procedures than using high-quality water. Wastewater is characterized by a high concentration of pollutants such as heavy metals, organic solvents, oil, xenobiotics, and other industrial wastes. Azolla can be used to reduce the concentration of wastewater pollution for agricultural use in such wastewater management.

A study on the growth of *A. microphylla* culture in domestic wastewater showed that Azolla acts as a biofilter to reduce the number of pollutants. Azolla live biomass acts as a potential bio accumulator for toxic pollutants, while dead biomass regulates pollutant concentrations through biosorption. In many higher aquatic plants, bioaccumulation is carried out by metalchelators, including phytochelatins, metallothioneins, organic acids, and amino acids. For this reason, Azolla species, which have the possibility of treating wastewater by bioremediation, have been widely studied (Table 2). There is great interest in the accumulation of heavy metals from aqueous solution by microbes and plants and thereby their purification (Elifantz *et al.*, 2002).

Azolla carries out the process of bioremediation by accumulating and biosorbing heavy metals. This means that heavy metals in wastewater can be controlled by Azolla's bioremediation pathway.

Based on the analysis of scientific sources, it was studied that the practical possibilities of algae not only in agriculture but also in environmental protection are very broad and promising. Although the processes of biosorption of chromium with the help of azoles are partially covered in scientific sources, it was noted that the processes of bioremediation of wastewater contaminated with chromium from leather tanning and processing enterprises with the help of azoles have not been studied in Uzbekistan.

Materials and Methods

Growth media of unicellular green algae

The following nutrient media were used to isolate and grow local unicellular green algae (microalgae) (Bozorova *et al.*, 2021; Safarov *et al.*, 2020): "Chu - 13" nutrient medium (g/l): KNO3 - 0.2, K2HPO4 - 0.04, MgSO4×7H2O - 0.1, CaCl2×6H2O - 0.08, iron citrate - 0.01, citric acid - 0.1, boron - 0.5 ppm, MnSO4×7H2O - 0.5 ppm, CuSO4×5H2O - 0.02 ppm, CoCl2×2H2O - 0.02 ppm, Na2MoO4×2H2O - 0.02 ppm, pH 7.5. "Tamiya" nutrient medium (g/l): KNO3 - 5, MnSO4×7H2O– 2.5, KH2PO4 - 1.25, FeSO4×7H2O - 0.003, EDTA - 0.037, the mixture of trace elements - 1 ml. Trace elements (g/l): H3BO3 – 2.86, MnCl2×4H2O – 1.81, ZnSO4×7H2O – 0.222, MoO3 – 176.4 mg/10 l, NH4VO – 229.6 mg/10 l (Safarov *et al.*, 2020).

Obtaining local microalgae aggregate cultures

To obtain collective cultures of local microalgae, water samples brought from Andijan regions were added to a sterile 100 ml "Chu-13" liquid nutrient medium and shaken at a temperature of 26-28C and a light of 3500-4000 Lk. The samples began to turn visually green in 14-20 days, allowing them to be seen under a light microscope. Stock cultures of microalgae were grown to a titer of 103 h/ml. After that, they were diluted up to 100 times and planted in 2% agar media, and grown at a temperature of 26-28C and light of 3500-4000 Lk (Waterhouse, 1993). The formed microalgae colonies were isolated by repeated cultivation in the liquid mineral nutrient medium.

Obtaining pure algological isolates

The methods used by Shakirov and Safarov in the selection and purification of microalgae were carried out in the order reflected in the scientific sources published by them. In particular, the suspension of microalgae isolates was diluted and spread on the top surface of the agar nutrient medium in a Petri dish using a sterile spatula and exposed to the light of 3500–4000 Lk until the growth of microalgae colonies. Grown microalgae colonies were collected using a microbiological hook and transferred to a liquid nutrient medium. These methods were repeated several times (6-8 times), and as a result, local unicellular green algae were isolated (Shakirov *et al.*, 2014).

Extraction of lipids from microalgae

The microalgae suspension was centrifuged at 3000 rpm for 20 min and the biomass was separated (Shakirov *et al.*, 2014). The amount of obtained biomass was measured on an analytical scale and dried at room temperature for 24 hours, and the obtained dry biomass was measured again. To grind dried biomass cells, 1 part of biomass was added to 3 parts of glass sand and ground in a porcelain mortar. The lipid extraction method of A.Ben-Amotz and T.G. Tornabene were modified to determine the amount of fat in biomass (Shakirov *et al.*, 2014).

Types of algae used, cultivation, and storage methods

Azolla caroliniana, *Lemna minor*, Pistia stratiotes, E. crassipes, which are common in the local conditions and are stored in the collection of the Tashkent Institute of Chemical Technology, "Biotechnology" laboratory, were used in the research work. The Tamiya nutrient medium was used as the basis for the processes of growing and maintaining these algae. Under laboratory conditions, modified organic nutrient media were maintained by adding weak concentrations of mineral salts. It is also widely used in scientific research as a nutrient medium for the cultivation of chromium-adapted cultures, the composition of which is Dwi P.Widiastuti (2017) (D) and Watanabe *et al.*, (1977) using recommended (W) feed media. Conditions for growing tall algae: water temperature 20C. The duration of cultivation in all samples is 7-14 days. The nutrient medium is a standard organic medium. pH was kept under control at around 6.0-8.0.

Determination of chromium in wastewater

Hexavalent chromium was used as a chromium research source. Hexavalent chromium-contaminated wastewater from a leather tanning and processing facility was also used in the study. Interstate standards (GOST 31956-2012) and the interstate standard approved by the Eurasian Council for Certification, Metrology, and Standardization (GOST 31956-2013) were used to determine the amount of chromium in wastewater.

Mass spectrometry

The chemical composition of the samples taken during the experiment was studied using a mass spectrometer. The mass spectrometer was used as described, the capillary temperature was 150°C, the capillary voltage was 35 V, and the tube lens voltage was 100 V. The m/z scan range was set to 50-500. Generally accepted methods were used in research (Guidelines (MUK 4.1.1482-03)., 2003).

Results and Discussion

Use of microalgae in Uzbekistan

Because the fishing industry in Uzbekistan is developing day by day and there is a need to further improve the nutritional base of the fishing industry, special attention is being paid to the preparation of live feed based on microalgae and its use in fisheries.

In particular, the formation of dry biomass of microalgae belonging to the genera *Ankistrodesmus*, *Botryococcus*, *Scenedesmus*, *Chlorella*, and *Chlorococcum* has been studied (Haydarov *et al.*, 2020a). According to the obtained results, microalgae *Ankistrodesmus* sp.15. strain produced 12.62% wet biomass, and an average dry matter yield of 3.35% was recorded. *Ankistrodesmus* sp.20 strain produced 11.36 g/l of biomass, and it was found that 3.24 g/l of dry biomass was released from it, which is 27.5% of the total mass of the cell (Fig. 1).

Botryococcus sp.5 of the genus *Botryococcus*. and *Botryococcus* sp. 4. strains produced 9.22-8.62 g/l of wet biomass, respectively, and 2.12-2.04 g/l of dry biomass was recorded. The average yield of dry matter from the total wet biomass was found to be 23.4%. *Scenedesmus* sp.7 of the genus *Scenedesmus*. and *Scenedesmus* sp.1. strains produced wet biomass in the amount of 11.44-10.66 g/l, respectively, and their dry biomass yield was 3.08-2.48 g/l, respectively.

It was found that the average yield of dry matter from the total cell biomass is 25.16%. *Chlorella* sp.3 of the genus *Chlorella*. strain produced 10.02 g/l wet biomass, while *Chlorella* sp.4. strain produced 9.22 g/l wet biomass. The dry mass yield of these strains was observed to be 2.56-2.48 g/l. It was noted that the yield of dry mass from the total wet biomass was 26.2%.

Chlorococcus of the *Chlorococcus* generation sp.4. while the strain produced 12.68 g/L of wet biomass, 3.64% G/L of dry biomass was found to be released from it. *Chlorococcus* sp of this generation. Strain 3, on the other hand, recorded a wet biomass output of 11.48 g/l, from which a biomass output of 3.01 g/l was recorded. Of the total biomass, the dry biomass output was recorded to be 27.5%. It is known that the process of obtaining dry biomass from wet biomass during the production of products determines industrial productivity and economic efficiency. During the studies, dry biomass output from wet biomass of microalgae was recorded at 27.6% dry biomass output from *Ankistrodesmus*, 25.2% from *Scenedesmus*, 23.4% from *Botryococcus*, 26.2% from *Chlorella*, and 27.5% from *Chlorococcus*.

The protein and fat storage of selected microalgae as subjects were studied in further research work (Fig. 2). According to the results of the study, it was noted that the protein and fat storage of microalgae differed sharply from each other in the Chu-13 nutrient medium. In particular, microalgae belonging to the genus *Ankistrodesmus* contain 43.2-46.4% protein and 27.4-32.2% fat (*Ankistrodesmus* sp.20; *Ankistrodesmus* sp.15). In the same nutrient environment, they can be seen that microalgae belonging to the genus *Scenedesmus* store up to 48.8-52.8% protein and 27.4-28.6% fat (*Scenedesmus* sp.7. and *Scenedesmus* sp.1).

According to the results of the study, representatives of the genus *Botryococcus* were recorded as microalgae genera that stored a relatively small amount of protein (46.2-46.8%) and fat (26.2-26.4%). During the research, it was noted that representatives of the genera *Chlorococcum* and *Chlorella* have high protein content (46.4-48.8%), but fat content (15.6-18.4%) is very low compared to all studied microalgae genera.

It is known that microalgae are an object that has its place and importance in almost all sectors of the economy. In particular, it serves as a productive resource for producing biodiesel, bioethanol, biogas, food and feed products, and the production of various agricultural biopreparations. Therefore, in obtaining microalgae biomass, the biomass produced and the release of dry mass from this biomass are of great importance.

Thus, in this research work, it was determined that microalgae produce biomass in the generation section, and dry mass comes out of this biomass.

Also, the dependence of protein and fat storage, which determine the nutritional value of microalgae, on the generations of microalgae was investigated. In particular, the protein and fat storage of microalgae in the Chu-13 nutrient medium differs dramatically from each other. It was noted that microalgae belonging to the genus *Ankistrodesmus* contain 43.2-46.4% protein and 27.4-32.2% fat. In the same nutrient environment, it can be seen that the microalgae belonging to the genus *Scenedesmus* contain 48.8-52.8% protein and 27.4-28.6% fat, respectively.

According to the obtained results, it was considered appropriate to use *Botryococcus* and *Chlorococcum* genera strains in obtaining feed with complete nutritional content for the aquaculture industry. Therefore, microalgae (*Botryococcus*, *Chlorococcum*), one of the crucial links in the food chain of zooplankton used as a live feed, were recommended as a natural food source in the artificial cultivation of zooplankton.

Use of macrophytes in Uzbekistan

After gaining independence (in 1991), our republic has gained a significant rating among exporters in the world as a result of the reforms implemented in the consumer industry sector, including the deep processing of leather raw materials. At the same time, consistent work is being done in our country in the field of environmental protection, rational use of natural resources, and improvement of sanitary and ecological conditions.

However, despite the ongoing reforms, measures aimed at maximum cleaning of the wastewater from leather processing plants from harmful chemicals are not sufficiently compelling. This requires using biological methods in harmony with the physicochemical methods widely used in wastewater treatment in production enterprises.

Although the importance of algae has been widely studied in the case of oil-gas, chemical, and oil-oil production enterprises based on algae in our country, scientific research work has not been carried out to reduce the amount of chromium in wastewater from leather processing enterprises.

Therefore, determining the place of algae in cleaning technical waters coming out of leather processing enterprises based on algae is of great scientific and practical importance.

Based on the analysis of scientific sources, it was studied that the practical possibilities of algae not only in agriculture but also in environmental protection are extensive and promising. Although the processes of biosorption of chromium with the help of azoles are partially covered in scientific sources, it was noted that the processes of bioremediation of wastewater contaminated with chromium from leather tanning and processing enterprises with the help of azoles have not been studied in Uzbekistan.

Therefore, in our next research work, we considered the possibility of purifying highly contaminated wastewater from tanning and processing enterprises of algae and practically studied the possibilities of chromium purification of algae. In preliminary experiments, the growth of higher plants was found in wastewater containing chromium. The obtained results are shown in Table 4.

According to the obtained results (Table 3), it was noted that the tolerance of higher algae to chromium is different. In our research work, when the tolerance of different types of higher algae to chromium was studied, it was observed that Azolla was somewhat resistant compared to the other higher algae in the experiment.

In particular, when *Lemna minor* L. was grown in a medium containing chromium at 3.0 mg/l for days, the viability indicators decreased by 26.79% on the second day compared to the first day, and by 52.6% on the third day compared to the second day, on the fifth day of observation and compared to the initial condition, it was noted that the incidence of necrosis or complete loss of viability was 96.79%.

Only 3.21% of the total viability was observed to retain viability on the fifth day of observation or to lose complete viability on the seventh day of observation.

Therefore, the culture of *Azolla caroliniana* isolated from the experimental variants that showed tolerance to chromium (+6) 3mg/l stress conditions (for seven days) was recommended to be used as a biological agent for chromium removal (Azimov *et al.*, 2022).

Azolla viability was recorded in hexavalent chromium water, which is the main raw material from the tanning and processing plant (Figure 3).

Table.1 Microalgae isolated from fishing lakes

№	Name of the source used	Distribution of source	Registration number of the International Gene Bank NCBI	Authors	Source
1	***Botryococcus braunii*-AnDI-115**	**Uzbekistan, Izboskan district, Andijan region**	**MZ148226**	**Sohibov B.O., Khujamshukurov N.A.**	**https://www.ncbi.nlm.nih.gov/nuccore/MZ148225**
2	***Tetradesmus obliquus*-AnDI-015**		**MZ148228**	**Sohibov B.O., Khujamshukurov N.A.**	**https://www.ncbi.nlm.nih.gov/nuccore/MZ148228**
3	***Scenedesmus quadricauda* -AnDI-44**		**MZ148229**	**Sohibov B.O., Khujamshukurov N.A.**	**https://www.ncbi.nlm.nih.gov/nuccore/MZ148229**
4	***Chlorococcum infusionum* –AnDI -76**		**MZ148226**	**Sohibov B.O., Khujamshukurov N.A.**	**https://www.ncbi.nlm.nih.gov/nuccore/MZ148226**
5	***Chlorococcum humicola*-AnDU- 03**	**Uzbekistan, Ulugnor district, Andijan region**	**MZ148227**	**Sohibov B.O., Khujamshukurov N.A.**	**https://www.ncbi.nlm.nih.gov/nuccore/MZ148227**

Table.2 Bioremediation of environmental pollutants by Azolla (Sela *et al.*, 1989)

Harmful Sources	Components	Azollaspecies	Scientific source
Heavy metals	**Cu, Cd, Pb, Ni, Cr, Hg, As, Au, Zn**	***A.filiculoides, A.microphylla, A.pinnata, A.caroliniana***	**Jafari *et al.*, 2010; Kanoun-Boulé *et al.*, 2009; Mufarrege *et al.*, 2010**
Oil products	**Diesel hydrocarbon, BTEX, oil**	***A.pinnata, A.africana***	**Cohen *et al.*, 2002; Edem *et al.*, 2010**
Pharmaceutical products	**Sulfadimethoxine**	***A.filiculoides***	**Forni *et al.*, 2006**
Paints	**Acid Red 88 (AR88)**	***A.microphylla***	**Padmesh *et al.*, 2005**

According to the obtained results, as the concentration of chromium in *A. caroliniana*, increases, the coefficient of development decreases ((mg/l)) 0,5→0,87%, 1,0→0,68%, 1,5→0,59%, 2,0→0,50%, 2,5→0,46%, 3,0→0,39%) and, correlatively, the corresponding decrease of the viability indicator was noted. When the obtained results were compared, it was found that dynamic growth was not observed on the 21st day of cultivation, and Azolla cultures could absorb chromium at the maximum level of 1.52 mg/g. Therefore, in the biological treatment of chromium-contaminated wastewater based on azolla culture, it is important to periodically update the biomass of cultures.

The use of macrophytes in the preparation of feed and feed additives

Macrophytes belonging to the family Lemnaceae are distinguished by their importance in various areas of the economy. In particular, it is an effective source in the production of food and feed products, and is widely used to clean wastewater and chemically contaminated water bodies from heavy metals and pesticides (Mkandawire *et al.,* 2007; Radić *et al.,* 2011). Therefore, in this research work, protein formation and amino acid composition of *Lemna minor* and *Lemna gibba* species from Lemnaceae macrophytes were determined, and it was recommended to use them as a source for the preparation of protein-rich feed supplement in the cultivation of *Tenebrio molitor*.

In Uzbekistan, when dry mass is released from the wet biomass of *Lemna minor* and *Lemna gibba*, dry abundance and its protein storage and amino acid content are analyzed, while *Lemna minor* produces a dry mass at an average of 5.21%, *Lemna gibba* has been found to have an average of 6.03%. It was noted that *Lemna minor* grown on a 1 m2 area had an average of 1429.83 g, and dry matter yield was 274.53 g, and *Lemna gibba* had an average of 1628.00 g, and dry mass yield was 268.91 g. Proteins in the dry mass were analyzed and it was noted that *Lemna minor* -16.10% and *Lemna gibba* synthesize 15.02% protein on average. The amount of essential amino acids in the purified protein of ryaska was analyzed. In this, *Lemna minor* threonine- 5.03; valine - 7.59; methionine - 0.39; isoleucine -5.83; leucine – 10.16; tyrosine - 2.73; phenylalanine was 6.21 and lysine 6.13 mg/100mg, while threonine was 4.75 in the case of pure *Lemna gibba* proteins; valine - 7.36; methionine - 1.23; isoleucine -5.74; leucine – 10.61; tyrosine - 4.32; phenylalanine was found to be 6.60 and lysine 6.13 mg/100mg. In the course of the research, it was concluded that the nutritional value of ryaska can be underestimated because there is minimal opportunity to obtain accurate data due to losses in the analysis of the protein content of the dry mass of ryaska and its amino acids. Therefore, to determine its actual nutritional value, it is appropriate to separate the pure protein from the dry mass and analyze the content of amino acids from this protein.

Table.3 Feed ration for rearing Tenebrio molitor F6 larvae, %.

Feed environment options	Components in feed	The ratio of components in feed, mg/100mg	The amount of protein in the feed, %
5-LWbA	***Lemna minor* L. flour**	**37,48**	**50,0**
	Wheatbran	**39,95**	
	***Azolla caroliniana* L. flour**	**22,57**	

Table.4 Chromium tolerance of some higher algae found in Uzbekistan

№	Macrophyte	Nutrient medium (chromium (+6) the content in water, 3.0 mg/l)					Control (artisan water of the enterprise)*				
		2	3	5	7	10	2	3	5	7	10
1	*A.caroliniana*	86,71±0,11	42,11±0,35	27,18±0,21	6,32±0,07	–	93,47±0,05	96,24±0,23	95,78±,041	98,43±0,33	99,6±0,36
2	*L.minor*	73,21±0,23	38,57±0,14	3,21±0,14	–	–	92,31±0,12	98,14±0,18	96,82±0,34	98,14±0,17	99,8±0,11
3	*P.stratiotes*	66,17±0,41	12,83±0,27	1,42±0,23	–	–	91,42±0,06	94,24±0,23	96,18±0,47	97,83±0,36	98,24±0,19
4	*E.crassipes*	42,14±0,18	7,14±0,32	0,68±0,12	–	–	86,23±0,14	88,41±0,23	92,63±0,28	96,17±0,13	99,4±0,23

Note: *the duration of the experiments is 5 times per species; NSR0.5– 0.05; The amount of chromium in the control water is negligible, 0.001 mg/l.*

Table.5 Indicators of the development of larvae of the genus *Tenebrio molitor* in the nutrient medium where the insect has stored it

+	F6 generation, number of larvae - 300	Average indicators of larvae					Average indicators of larvae		
		Initial weight, mg	Final weight, mg	Growth rate, %	Protein, %	Fat, %	Protein, %	Fat, %	Weight, mg
Added insect flour quantity - 10%									
5-LWbA	TMO-2	4,16	94,81	93,81	64,50	26,48	64,49	26,73	94,00
5-LWbA	TMO-6	4,08	95,19	94,19	64,47	26,98			
Added insect flour quantity - 15%									
5-LWbA	TMO-2	4,16	101,33	98,84	64,26	28,56	64,42	28,65	98,89
5-LWbA	TMO-6	4,08	102,46	98,94	64,58	28,75			
Added insect flour quantity - 20%									
5-LWbA	TMO-2	4,16	101,28	92,22	64,22	28,10	64,23	28,34	101,18
5-LWbA	TMO-6	4,08	101,08	99,50	64,24	28,58			
Added insect flour quantity - 25%									
5-LWbA	TMO-2	4,16	100,98	99,43	64,03	28,04	64,08	28,33	101,01
5-LWbA	TMO-6	4,08	101,04	99,65	64,13	28,61			

Note: 5-LWbA (according to table 3, 100 mg): Protein content in food-50%; the Moisture content of food medium - 8.2%; Relative humidity -75%; n-5; P5.0= 0.01; diapause: 8:16. The maximum duration of larval development is 35 days.

Fig.1 Dry biomass output from wet biomass in microalgae generation cross-section (%), n=3

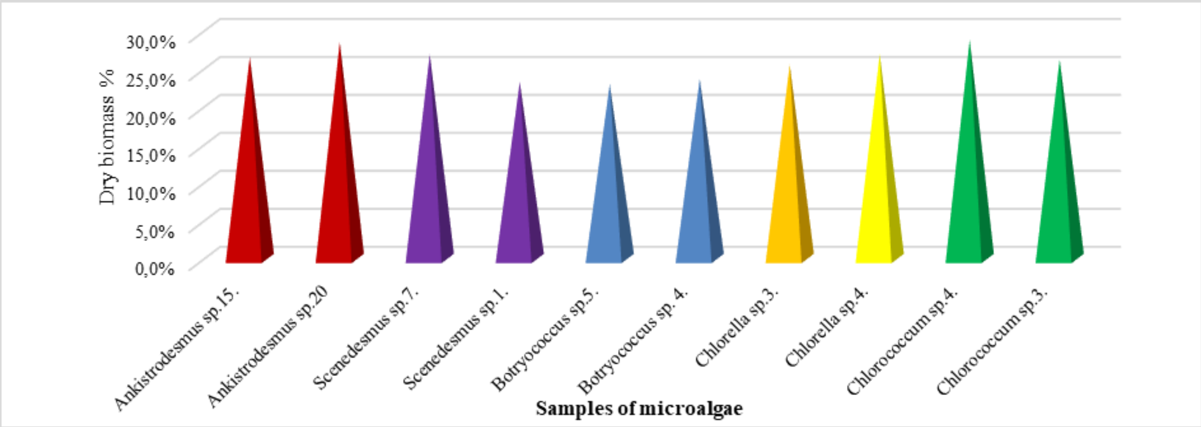


Fig.2 Production of protein and fat by microalgae genera (relative to dry matter, %), n=3

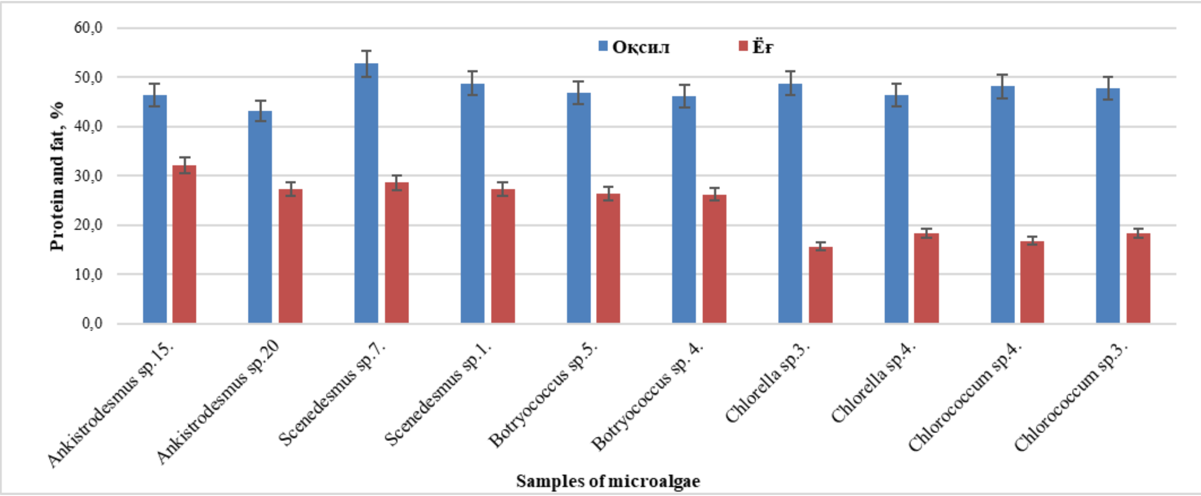
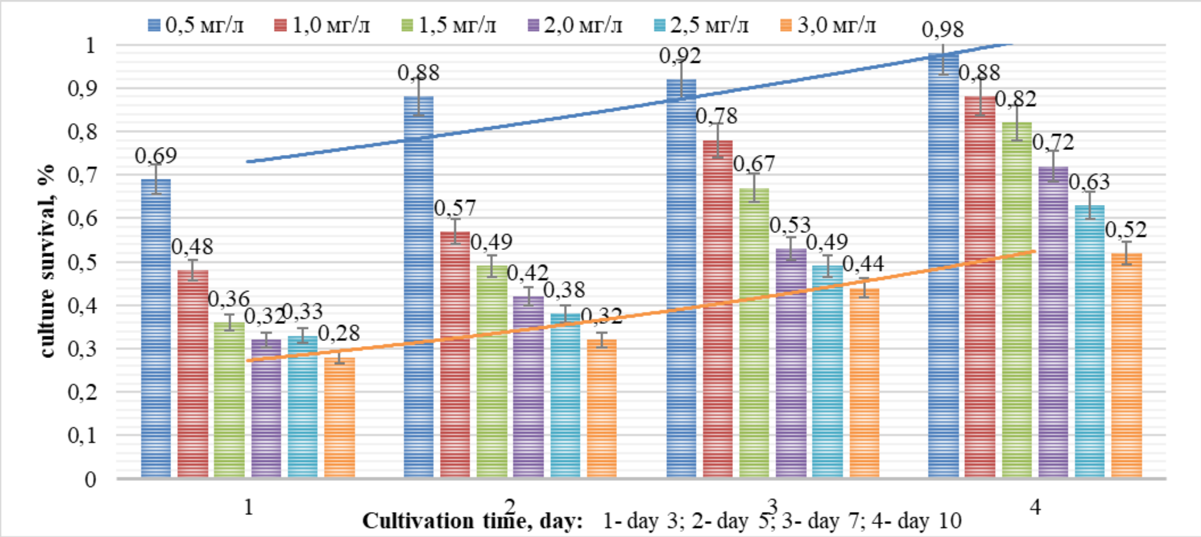


Fig.3 Viability of Azolla at various concentrations of chromium, %



In the course of our research, nutrient mediums with new compositions were developed based on mixtures of *Lemna minor* L. flour (protein - 16.10%), wheat bran (protein - 14.2%), and Azolla Carolina L. (protein - 27.6%). For the first time, the theory "Insect to insect" was put forward and its scientific basis was shown. In particular, for the cultivation of *Tenebrio molitor* larvae under controlled conditions, it was recommended to use a nutrient medium with a new composition prepared by adding 5-LWbA to the nutrient medium in the amount of 15% of the larval meal of *Tenebrio molitor* larvae grown for 35 days.

Also, as a result of the implementation of the technology of industrial cultivation of *Lemna minor* macrophyte, the composition of expensive feed products such as wheat flour, wheat bran, corn flour, soybean meal, used for the cultivation of nutritious insects, and macrophytes, which are non-traditional sources of food, have been improved based onAzolla and small ryaska. As a result, a new composition of nutrient medium was developed (5-LWbA) that ensures the optimal development and reproduction of the feeding insect *Tenebrio molitor* L., which made it possible to replace conventional feed products with non-traditional sources by up to 60% and saves the main feed products used by up to 26.7%. Based on the developed 5-LWbA nutrient medium, a prototype technology for growing biomass up to 30 tons per year based on *Tenebrio molitor* L. was developed and implemented in the fishery (Table 5).

As a result, it was possible to increase the growth rate of *Tenebrio molitor* larvae to 88.82%, the protein content to 64.80%, and the fat content to 28.65% based on the new nutrient diet. Based on 5-LWbA nutrient medium, fry was fed, and 30 tons of fish were grown on the farm. Compared to the control option, 1,933 kilograms more fish were produced in the experimental option compared to the total productivity. The periodic application of this feed composition in other stages of the fish-feeding process creates the possibility of achieving a high level of productivity.

Acknowledgments

We express our gratitude to the head of the Laboratory of Microwine making of the Research Institute of Plant Genetic Resources and Professor of the Department of Biotechnology of the Tashkent Institute of Chemical Technology prof. N. A. Khujamshukurov

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How to cite this article:

Xurshidbek O. Abdullayev, Mukhlisa Nigmatullayeva, Feruzakhon N. Abdikholikova, Davronjon H. Saminjonov, Saidkamol Xaydarov, Dilafruz Kh. Kuchkarova, Baxriddin O. Soxibov and Nortoji A. Khujamshukurov. 2023. Problems and Prospects of the Use of Macrophytes and Microalgae in Sectors of the Economy (Scientific Analysis on the Example of the Republic of Uzbekistan and Countries of the World). *Int.J.Curr.Microbiol.App.Sci.* 12(07): 1-15. **doi:** https://doi.org/10.20546/ijcmas.2023.1207.001