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Study of the possibility of using agricultural wastewater for the accumulation of microalgae biomass

Abstract. The article examines the possibilities of using various agricultural wastewater for the accumulation of biomass of microalgae Chlorella vulgaris SP BB-2, Parachlorella kessleri Bh-2 and Chlamydomonas reinhardtii Dangeard CC-124. As a result of the study, a strain of microalgae C.vulgaris SP B-2 was selected for use in the treatment of fishery wastewater with the possibility of obtaining feed biomass. According to the research results, this strain showed an increased growth rate in aquaculture wastewater, also the effectiveness of reducing the COD index, removing ammonia and phosphates. According to the results of the study, the protein content in the resulting biomass of C-vulgaris SP BB-2 was 57.0 ± 1.2%, lipids 16 ± 1.2% and carbohydrates 11.4 ± 1.4% when grown on wastewater. The strain of C.vulgaris SP BB-2 can be effectively used in the bioremediation of wastewater in order to purify it from organo-mineral contaminants.
Key words: wastewater, bioremediation, microalgae, biomass.

Introduction

Protection of water resources from depletion, pollution and their rational use for the needs of the national economy is one of the most important problems requiring urgent solutions. Currently, environmental protection measures are being widely implemented in many countries, in particular on the rational use of water resources. The agricultural industry is directly connected with the use of water resources and imposes very high requirements to their mode, quantitative and qualitative condition. This fast-growing industry also leads to the production of a huge amount of wastewater and has a number of negative consequences for the environment and the economy. Wastewater from agriculture, including animal husbandry, consists of nitrogenous components such as ammonium, nitrite, nitrate, phosphorus and organic carbon [1]. The discharge of polluted agricultural wastewater into a clean body of water can cause eutrophication of natural ecosystems. To improve the economic prospects and sustainability of the agricultural sector, it is important to focus on wastewater treatment and reuse. Many methods have been developed for agricultural wastewater treatment, such as the denitrification process used to release nitrogenous compounds into the atmosphere [2], chemical precipitation used to remove phosphorus using iron chloride [3]. These methods are expensive and also produce toxic compounds as by-products [4].

Bioremediation of agricultural wastewater by cultivation of microalgae is a promising method of purification. This method can be cost-effective, environmentally friendly, and also produce valuable biomass based on microalgae. The cultivation of algae in wastewater provides a double advantage, such as wastewater treatment and the production of cellular biomass rich in lipids, proteins, carbohydrates and many other products [5]. Microalgae use the nutrients present in wastewater and convert them into biomass, thereby can improve the economy of microalgae biomass production [6]. Microalgae also enrich the aquatic environment with oxygen due to photosynthesis, thereby accelerating oxidative processes and mineralization of organic impurities. In addition, many microalgae are able to feed not only on minerals, but also on simple organic compounds available in wastewater [7]. The use of active microalgae strains in wastewater provides an opportunity to obtain cheap microalgae biomass with high nutritional value for animals [8]. Microalgae are the natural food of the aquaculture ecosystem and they are widely used for larvae, crustaceans and mollusks [9]. Microalgae-based feeds can provide the necessary proteins, essential amino acids, fatty acids, pigments, etc. for high-quality livestock products. The use of wastewater rich in bioorganic compounds as nutrient media for the cultivation of microalgae is very promising, since it allows combining technologies for biological purification of water resources and the accumulation of microalgae biomass, which is a valuable raw material for the production of various industrial products. In connection with the above, it becomes obvious the relevance of the study of the possibility of waste-free use of microalgae in wastewater treatment with the production of biomass, which is of potential interest in agriculture. The purpose of this work was to study the possibility of using various agricultural wastewater for the accumulation of microalgae biomass.

Materials and methods

The objects of study were a strain of microalgae *Chlorella vulgaris* SP BB-2 isolated from the polluted lake Bilikol, located in the south of the Republic of Kazakhstan (*42°59'18"* n.l., *70°41'14"* e.l.) and strains of microalgae *Parachlorella kessleri* Bh-2 and *Chlamydomonas reinhardtii* Dangeard CC-124 from the collection of the Al-Farabi Kazakh National University. The selected strains from the collection were characterized as undemanding for the use of specific nutrients during cultivation [10].

Cultivation of microalgae in laboratory conditions and determination of growth rate and biomass.

Microalgae strains were cultured under laboratory conditions in three mediums:

a) laboratory medium (control): Tamiya medium for *C.vulgaris* SP BB-2 and *P.kessleri* Bh-2, L2-min medium for strain *C.reinhardtii* CC-124 [10];

b) wastewater samples taken from the reservoir of the warm-water fish fishery (tilapia) located in the village of Saimasai, Almaty region (42°44'72" n.l., 77°33'06" e.l.). 25.12 mg/l of ammonia, 3.8 mg/l of nitrites, 16.07 mg/l of nitrates and 5.13 mg/l of phosphates were found in the wastewater of the reservoir. Biochemical oxygen demand (BOD5) was 8.5 mg/O_2/l and chemical oxygen demand (COD) was 96 mg/O_2/l.

c) wastewater samples taken from the reservoir of the Alatau-Kus poultry farm (43.478297253339015, 76.85432846049972.), in the village of Boraldai, Almaty region. The composition of wastewater from the Alatau-Kus poultry farm: ammonia 50.0 mg/l, nitrite 5.2 mg/l, nitrate 18.6 mg/l, phosphate 8.8 mg/l. Biochemical oxygen demand (BOD5) was 25 mg/O_2/l and chemical oxygen demand (COD) was 990 mg/O_2/l.

Microalgae were previously grown for 8-10 days on appropriate nutrient media in conical flasks with a volume of 250-1000 ml under round-the-clock artificial lighting with Flora Led 35 D120 lamps (80 micromole photons · m^{-2} · s^{-1},) and at a temperature of 25°C. Monitoring of the growth rate of microalgae in culture was carried out on the basis of accounting for changes in their abundance using the Goryaev chamber [10].

In experiments on wastewater treatment, a rectangular laboratory installation with a volume of 10 liters (35×12×25 cm) was used. Cultivation of microalgae was carried out for 14 days when bubbling with air with 1.5% CO2 (bubbling rate 0.3 l/min), at a temperature of 25 ° C and illumination of 80 micromole photons · m^{-2} · s^{-1}. The initial number of microalgae cells was 0.5×106 cl/ml in all experimental variants.The productivity of the studied microalgae strains was determined by the accumulation of dry weight of biomass in accordance with the standard method for the total amount of suspended solids [11].

Analysis of changes in ion concentration in the medium during cultivation of microalgae

Chemical oxygen demand (COD) was determined by measuring the oxidability of potassium bichromate, registering the optical density of samples at λ=600 nm on a PD-303UV spectrophotometer (Japan) [12].

The content of ammonium nitrogen (NH_4^+) was determined using a Nessler reagent by measuring the optical density of samples at λ=414 nm in accordance with the methods described in (Clesceri et al., 1998). Phosphorus concentration in the form of orthophosphate (PO_4^{3-}) was determined at 610 nm using ammonium molybdate and malachite green on a *PD-303UV* spectrophotometer (Japan) [13].

Analysis of the biochemical composition of the resulting biomass

The total protein content was determined using the Lowry method [14]. Carbohydrates were determined according to [15], and lipids – according to [16]. The optical density of the studied samples was measured using a *PD-303UV* spectrophotometer (Japan). The percentage of proteins, carbohydrates and lipids was determined relative to the weight of dry biomass of microalgae.

Assessment of the toxicity of microalgae biomass using the D. magna test object.

The toxicity of microalgae biomass was determined in accordance with GOST 4173:2003. Crustaceans-Daphnia (Daphnia magna Straus) were used as test objects. The method is based on establishing the difference between the number of dead daphnia in the analyzed sample (experiment) and control. The criteria of acute lethal toxicity is the death of 50% and more of daphnia in the experiment for 96 hours of biotesting. In determining acute toxicity, the criteria was the mortality of test organisms in relation to control. The laboratory culture of daphnia was carried out on well-maintained tap water and tested for sensitivity before the experiment. To test acute toxicity, young daphnia under the age of 24 hours were used. 100 ml of water was poured into the cups and the microalgae biomass was diluted with cultivation water to a concentration of 0.5 – 0.6 units of optical density. The repetition is threefold. 30 young daphnia were placed in each cup and exposed under optimal conditions for 96 hours. The account of the surviving daphnia was carried out after 1, 6, 24, 48, 72 and 96 hours.

All measurements were carried out in at least five repetitions. The figures show the data of average values and standard deviations. Statistical processing of the obtained data was carried out using single-factor analysis of variance (ANOVA) with a confidence level of 95%.

Results and discussion

Selection of microalgae strain with high growth values.

Cultivation of microalgae on agricultural wastewater is a very promising approach for bioremediation, however, not all types of microalgae are able to grow on wastewater. Therefore, fast-growing microalgae strains deserve special attention in the context of wastewater bioremediation. Equally important are such characteristics as the ability to mixotrophic growth, tolerance to adverse factors, high rate of cell subsidence, and the content of valuable substances in biomass. At the first stage, screening was carried out in order to select a strain with high growth rates in wastewater.

In accordance with the results presented in Fig. 1, the dynamics of the growth of *C.vulgaris* SP BB-2 cells in the wastewater of the fishery showed a high growth compared to the control. The number of cells of this strain on the 6th day of cultivation in wastewater was 6.3 × 106 cells/ml. The growth rate of *C.vulgaris* SP BB-2 microalgae cells on Tamium medium was 3.8 × 106 cl/ml. And on the water from the poultry farm, the growth dynamics of microalgae for the first day showed higher indicators compared to the control, and from day 6 the growth dynamics on the contrary decreased and the growth of *C-vulgaris* SP BB-2 was 4.1 × 106 cells/ml. The low growth rate of microalgae may be due to the high content of biogenic elements (Fig.1 A). Thus, according to the results of studying the growth of microalgae by the number of cells, it can also be argued that the strain of *C.vulgaris* SP BB-2 is most actively developing in the wastewater of aquaculture.

According to the results, the study of *Parachlorella kessleri* Bh2 and *Chlamydomonas reinhardtii* C-124, showed lower growth rates in aquaculture wastewater compared to the control, but maintaining growth dynamics for twelve days (Fig.1 B, C). Poultry farm wastewater turned out to be an unfavorable nutrient medium for all three studied strains due to high indicators concentrations of biogenic elements. In all three strains, the growth dynamics grew intensively in the lag phase, then the transition of microalgae from the logarithmic phase to the phase of slowing growth and dying was observed. High concentrations of nutrients in wastewater limit the penetration of light due to turbidity and often lead to toxicity, negatively affecting the growth of microalgae. To solve these problems, dilution is usually required before being used as a medium for microalgae growth. But there is a lot of literature data that poultry farm wastewater is a good nutrient medium for growing microalgae in a diluted form [17]. Aquaculture wastewater, on the contrary, contains an intermediate set of all nutrients and therefore can be used directly for the cultivation of microalgae.

The fact of the high growth rate of the *C.vulgaris* SP BB-2 strain in wastewater was confirmed by the analysis of biomass growth (Table 1). When growing *C.vulgaris* SP BB-2, *P.kessleri* Bh-2 and *C.reinhardtii* CC-124 strains in poultry farm water, low biomass growth was observed with values of 1.07, 1.05 and 1.06 g/l, respectively. While the biomass gain on laboratory media was 2.47 g/l for the strain *C.vulgaris* SP BB-2, 2.45 g/l for *P.kessleri* Bh-2 and 2.42 g/l for *C.reinhardtii* CC-124. When growing aquaculture from the studied microalgae strains on wastewater, the highest biomass productivity was shown by the strain *C.vulgaris* SP BB-2. The results obtained show that aquaculture wastewater can be effectively used as nutrient media for the accumulation of biomass of *C.vulgaris* SP BB-2 microalgae.

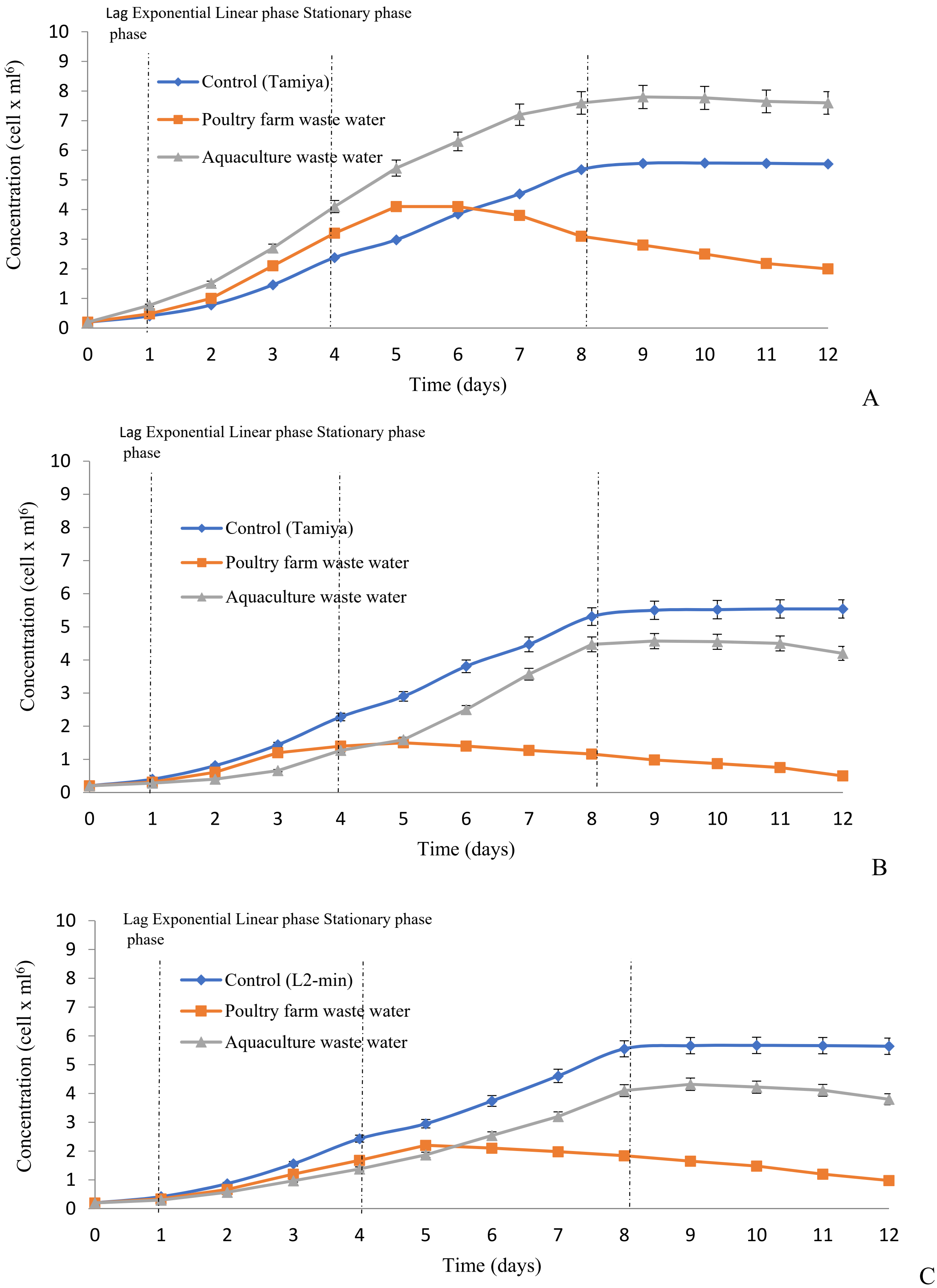

Figure 1 – Growth dynamics of microalgae strains during cultivation in wastewater
(1 control option – Tamiya medium for *Chlorella vulgaris* sp. BB2*, Parachlorella kessleri* Bh-2, L2-minimum medium for *Chlamydomonas reinhardtii* CC-124; 2 option fish farm wastewater; 3 option poultry farm wastewater,
A – *Chlorella vulgaris sp.* BB-2, B – *Parachlorella kessleri* Bh-2, C – *Chlamydomonas reinhardtii* C-124.

Table 1 – Accumulation of biomass of microalgae strains during cultivation on various wastewater, for 14 days

Microalgae cultivation media	Biomass yield,g/l		
	C. vulgaris SP BB-2	*P. kessleri* Bh-2	*C. reinhardtii* CC-124
Laboratory environments	2.47±0.01	2.45±0.01	2.42 ±0.01
Aquaculture waste water	2.89±0.01	2.38 ±0.01	2.25±0.01
Poultry farm waste water	1.07±0.03	1.05±0.01	1.06±0.01

Efficiency of aquaculture wastewater treatment with microalgae

The efficiency of removing pollutants is an important criterion for choosing a strain of microalgae in wastewater treatment. Basically, this process is associated with the consumption of organic carbon, nitrogen and phosphorus from the medium [18]. To assess the effectiveness of microalgae strains in the treatment of aquaculture wastewater, such indicators as COD (chemical oxygen demand), the concentration of ammonium nitrogen and phosphorus were measured. According to the results of the study, all microalgae cultures have shown potential in the treatment of aquaculture wastewater from organic and biogenic substances during cultivation.

COD is an indirect indicator of the presence of organic matter in wastewater. It follows from the data in Fig. 2 that in the presence of all microalgae strains there was a decrease in COD. But the strain of *C. vulgaris* SP BB-2 showed the greatest effectiveness.

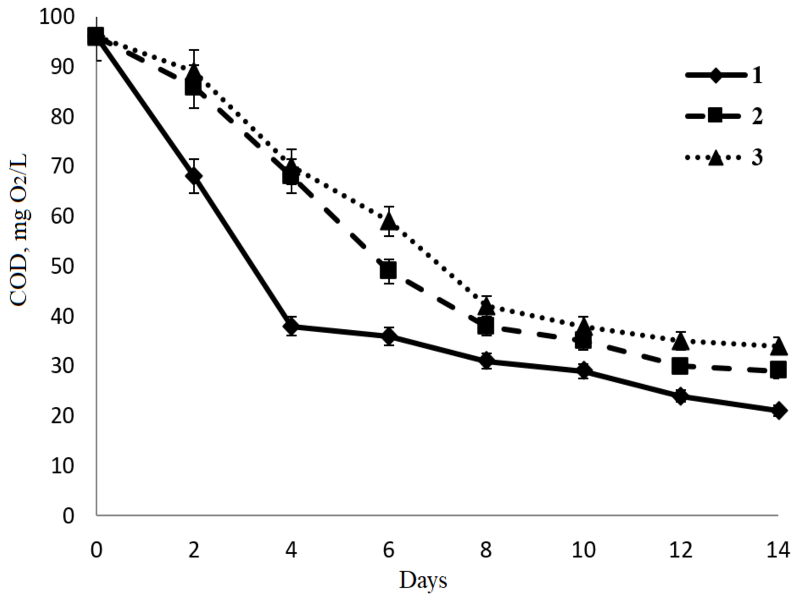

Figure 2 – Dynamics of the COD index during cultivation of microalgae strains *C.vulgaris* SP BB-2 (1), *C.reinhardtii* C-124 (2) and *P.kessleri* Bh-2 (3) on aquaculture wastewater.

Microalgae are able to absorb nitrogen in the form of ammonia, nitrite, nitrate and urea, but ammonium nitrogen is more preferable [19-20]. We studied the consumption of ammonium nitrogen by microalgae strains from aquaculture wastewater (Fig. 3A). In all microalgae strains, high activity of consumption of the ammonium form of nitrogen was observed. Its almost complete removal from the medium was achieved on the 8th day of growth in all studied strains.

Phosphorus is also another important trace element that affects the growth of microalgae [21]. The change in phosphorus concentrations in the form of orthophosphate is shown in Fig.3B. The consumption of phosphorus in the form of orthophosphate in the first six days of cultivation in all strains was comparable and amounted to >75% of the initial value (Fig. 3B). By the end of cultivation on day 14, this indicator for *C.vulgaris* SP BB-2 was 100%. The results obtained show that all microalgae strains have a high rate of removal of ammonia and phosphates from aquaculture wastewater, however, *C.vulgaris* SP BB-2 shows great efficiency.

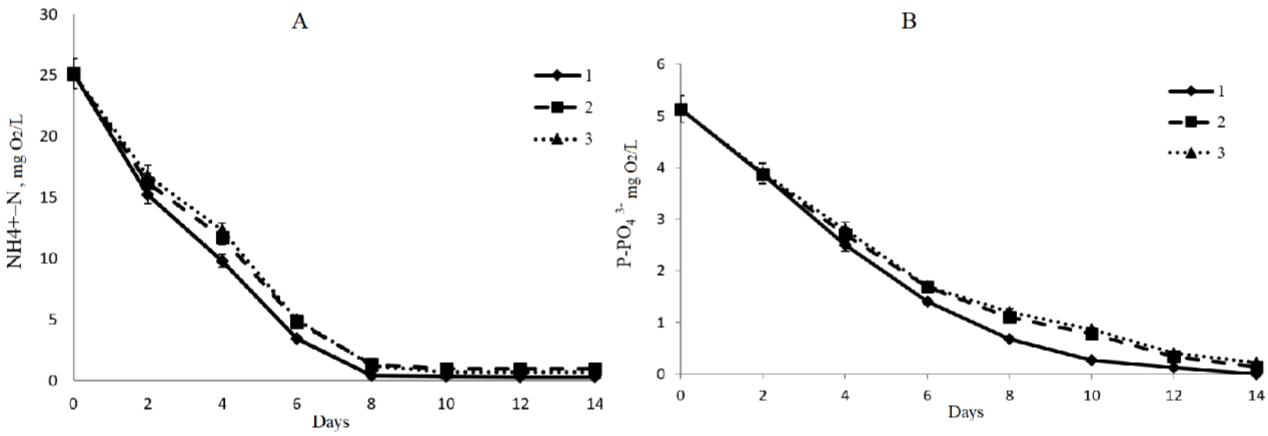

Figure 3 – Reduction of NH_4^+ (A) and PO_4^{3-} (B) concentrations during cultivation of *C.vulgaris* SP BB-2 (1), *C.reinhardtii* CC-124 (2) and *P. kessleri* Bh-2 (3) microalgae strains on aquaculture wastewater.

Biochemical composition of microalgae biomass

Microalgae attract attention as a potential raw material source for food and feed purposes [22]. We analyzed the composition of the main bio-organic components of the resulting biomass after cultivation in wastewater. According to the results of the study, the protein content in the obtained biomass of *C.vulgaris* SP BB-2 was 57.0 ± 1.2%, for *C.reinhardtii* CC-124 – 35.55 ±1.1% and for *P. kessleri* Bh-2 – 32.3±1.1% (Table 3). The lipid content for *C.vulgaris* SP BB-2 was 16 ± 1.2% and for *C.reinhardtii* CC-124 and *P. kessleri* Bh-2 – 15±1.2% when grown on wastewater. The indicators of the total carbohydrate content for all strains were similar (Table 3). Thus, the *C. vulgaris* SP BB-2 strain showed a high protein content when cultured in wastewater, which makes it possible to recommend the use of these microalgae for feed purposes.

Table 2 – The content of proteins, carbohydrates and lipids in the biomass of different strains of microalgae after cultivation in aquaculture wastewater for 14 days (% of dry weight)

Parameters, %	*C. vulgaris* SP BB-2	*P. kessleri* Bh-2	*C. reinhardtii* CC-124
Protein	57.0±1.2%	32.3± 1.1%	35.55± 1.1%
Carbohydrate	11.4 ± 1.4%	11.4± 1.2%	11.56± 1.1%
Lipid	16 ± 1.2%	15± 1.2%	15± 1.2%

The active use of microalgae as a bioremediation of wastewater helps to purify water from excessive amounts of mineral and organic compounds. Microalgae capable of mixotrophic growth are usually cultivated in wastewater, since in this case parallel utilization of organic carbon sources is possible [23].

By releasing oxygen during photosynthesis, microalgae ensure the oxidation of organic pollutants in the environment. In addition, microalgae are able to assimilate nitrogen and phosphorus from wastewater, which compares favorably with conventional remediation methods. One of the approaches to solving the problem of wastewater treatment is the choice of highly active microalgae strains [24].

Assessment of the toxicity of microalgae biomass using the D. magna test object.

Biotesting using daphnia D. magna is widely used in world practice to determine the toxicity of technogenic and natural environments. Daphnia belongs to the "classic" test objects. [25].

When assessing the toxicity of microalgae biomass by the survival rate of daphnia at 96-hour exposure, it was found that all the studied microalgae cultures did not have a negative effect on crustaceans. The survival rate in all variants of the experiment was 100 % . The deviation from the control was no more than 10%, i.e. the samples were non-toxic for this test object.

In our work, a strain of *C. vulgaris* SP BB-2 microalgae was selected for use in the treatment of fishery wastewater with the possibility of obtaining feed biomass. According to the research results, this strain showed an increased growth rate in the wastewater of aquaculture. This strain also showed the effectiveness of reducing the COD index, which is used as an indirect indicator of the amount of organic compounds present in wastewater, and its decrease indicates that microalgae may have the potential to use organic compounds [26]. A high rate of COD reduction is an indicator of the viability of microalgae at initially high levels of COD [27]. This strain has also demonstrated efficacy in removing phosphates from aquaculture wastewater.

The use of wastewater as nutrient media for the cultivation of microalgae is very promising, since it allows combining technologies for the purification of water resources and the accumulation of microalgae biomass. In the practice of aquaculture, microalgae are important sources of fish nutrition for direct consumption or as indirectly prepared feed. Microalgae biomass is rich in proteins, lipids and carbohydrates, which are essential nutrients for aquatic animals. Currently, there is a lot of literature data on studies of microalgae cultivation processes using wastewater [28]. However, it is noted that not every strain of microalgae is able to adapt to growth in wastewater [29]. The results of our research have shown that the strain of *C.vulgaris* SP BB-2 has the best adaptive ability to grow in the wastewater of fisheries reservoirs from all the studied microalgae strains. Strain *C.vulgaris* SP BB-2 can be effectively used in bioremediation of wastewater in order to purify it from organo-mineral contaminants. In addition, this strain has shown a high accumulation of biomass with an increased protein content, and therefore can be recommended as a by-product for obtaining a cheap feed additive in aquaculture.

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