Reproduction of chlorella (chlorella vulgaris) under laboratory conditions and its application in fish farming

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Abstract. This study investigates the reproduction dynamics of *Chlorella vulgaris* under controlled laboratory conditions and evaluates its application in enhancing aquaculture productivity. Pure cultures of *Ch. vulgaris* were propagated in photobioreactor systems under optimized conditions involving varied light intensities, pH ranges, and nutrient concentrations. Reproductive performance was assessed through growth rate, chlorophyll content, cell density, and autosporulation analysis. Maximum biomass yield was obtained at 25°C, pH 7.5, and light intensity of 150 µmol photons m^{-2} s^{-1} using nitrogen-enriched media. Harvested biomass was incorporated into experimental aquafeeds at 5% and 10% inclusion levels, and its effect on the growth performance, feed efficiency, and water quality in *Oreochromis niloticus* (Nile tilapia) was assessed over a 45-day period in recirculating aquaculture systems. Results demonstrated that *Ch. vulgaris* supplementation significantly improved specific growth rate (SGR), feed conversion ratio (FCR), and fish survival compared to control groups. Furthermore, live microalgae addition contributed to reduced ammonia and nitrate levels, confirming its biofiltration capacity. This study highlights the dual role of *Chlorella vulgaris* as a nutrient-rich feed additive and an eco-functional agent in aquaculture. The findings support the integration of laboratory-optimized *Ch. vulgaris* into sustainable fish farming systems and provide a scalable model for enhancing productivity while improving environmental resilience.

1 Introduction

By 2050, the world's population is expected to grow by a third (2.3 billion people), which will require an increase in food production of approximately 70%. Microalgae are photosynthetic microorganisms that are characterized by rapid growth, production of compounds of high biological value, and adaptability to various environmental conditions. They can often be grown using wastewater, industrial effluents, or inexpensive nutrients, which not only allows for efficient biomass production but also protects the environment

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from pollution. Therefore, the use of microalgae in sustainable development strategies is becoming increasingly important[1]. Green algae also play an important role in addressing environmental issues. This is because they reduce pollution by absorbing excess nutrients from the environment and contribute to environmental sustainability. [2] Green algae, which play an important role in global oxygen production and the carbon cycle, offer opportunities for use in biofuel production, animal feed, pharmaceuticals, cosmetics, and the food industry [3].

However, one of the most impactful and scalable uses of *Ch. vulgaris* is within the aquaculture industry, particularly as a dietary supplement, water quality enhancer, and natural probiotic agent in fish farming systems[4,5].

The continuous expansion of the global aquaculture sector has led to challenges in maintaining ecological balance and optimizing feed formulations. Traditional fish feed ingredients, such as fishmeal and soy protein, are not only expensive but also contribute significantly to environmental degradation. In contrast, microalgae like *Chlorella vulgaris* offer a renewable and ecologically responsible alternative. Rich in proteins, essential amino acids, polyunsaturated fatty acids, vitamins, and pigments such as chlorophyll and carotenoids, *Ch. vulgaris* presents itself as a natural bio-factory capable of improving fish health, growth performance, and stress resistance. Furthermore, its intrinsic ability to sequester nitrogenous waste and heavy metals from water environments aligns well with the ecological needs of closed-loop and recirculating aquaculture systems (RAS)[6,7].

To exploit these benefits at an industrial level, understanding and controlling the reproduction of *Chlorella vulgaris* under laboratory conditions is crucial. Laboratory cultivation allows researchers to explore the optimization of growth parameters, including temperature, light intensity and wavelength, pH, nutrient concentrations, and CO_2 enrichment. This foundational knowledge is vital not only for upscaling biomass production but also for maintaining culture stability and maximizing the algal bioactivity. Despite the extensive research on microalgae over the past two decades, species-specific studies on *Ch. vulgaris* reproduction dynamics and their direct application in aquaculture remain fragmented and underdeveloped[8].

Cultivation systems ranging from batch cultures to photobioreactors have been tailored to exploit these biological responses, enabling the controlled mass production of *Ch. vulgaris* for aquaculture applications.

The integration of *Ch. vulgaris* in fish farming is a multi-pronged intervention. First, its role as a direct feed supplement, particularly for larval and juvenile stages, addresses nutritional deficits that are common in early fish development. Microalgae provide a naturally digestible form of nutrients that stimulate enzymatic activity and bolster immunity. In larviculture, where feed particle size is a limiting factor, *Ch. vulgaris* offers an optimal micro-size profile along with high palatability. Second, the presence of bioactive compounds such as β-glucans and antioxidants in *Ch. vulgaris* has demonstrated immunostimulatory effects, reducing the prevalence of bacterial and fungal infections.

Green algae, which can grow rapidly using CO2 and light, account for 50-87% of global oxygen production. Bioactive substances (carotenoids, chlorophyll, polyphenols, etc.) have antioxidant, antibacterial, and antitumor properties, and their use as supplementary feed for livestock, poultry, and fish strengthens immunity and improves product quality. Therefore, species such as Chlorella, Spirulina, Dunal-iella, and Haematococcus are widely cultivated on an industrial scale.

Nevertheless, several challenges must be addressed before large-scale adoption of *Ch. vulgaris* in fish farming becomes mainstream. These include the high cost of downstream processing, risks of contamination in open culture systems, and the need for standardized protocols for biomass harvesting, drying, and incorporation into feed. Regulatory approval and consumer perception also play significant roles in determining the acceptability of microalgae-based aquafeeds. Therefore, a multidisciplinary approach involving microbiologists, aquaculturists, engineers, and policy-makers is essential to realize the full potential of *Ch. vulgaris* in aquaculture

The reproduction of *Chlorella vulgaris* under laboratory conditions and its subsequent application in fish farming represent a frontier of innovation in sustainable aquaculture.

2 Materials and methods

This study was conducted in two integrated phases: (i) laboratory cultivation and reproduction optimization of *Chlorella vulgaris* under controlled environmental conditions, and (ii) experimental application of the obtained biomass in a model aquaculture system for evaluating its effects on water quality and fish growth performance. All procedures were carried out at the Microalgae Biotechnology Laboratory and Experimental Aquaculture Unit of [Name of Institution], following biosafety and animal care guidelines.

Microalgae Strain and Inoculum Preparation

A pure culture of *Chlorella vulgaris* (strain ID: C.v-BUK-22) was obtained from the algal culture bank of the [Institution or Research Center]. The strain was maintained on Bold’s Basal Medium (BBM) under sterile conditions and sub-cultured weekly to ensure active growth and purity. Initial inocula were standardized at 1×10^6 cells/mL using a hemocytometer and spectrophotometric calibration at 680 nm (OD_{680}).

Culture Conditions and Reproduction Optimization

Microalgal cultures were maintained in 2 L Erlenmeyer flasks and flat-panel photobioreactors (5 L working volume) under strictly regulated conditions. Temperature was maintained at 25 ± 1°C using a digital incubator. Continuous aeration (filtered ambient air enriched with 2% CO_2) was supplied at a flow rate of 1.5 L/min. Lighting was provided via full-spectrum white LED panels at a photon flux density of 150 µmol photons m^{-2} s^{-1}, controlled through programmable timers (16:8 h light: dark cycle).

To optimize reproduction, a factorial design was employed to assess the effect of varying nutrient concentrations (N:P:K), pH (ranging from 6.5 to 8.5), and light intensities (100–300 µmol m^{-2} s^{-1}) on growth rate and biomass yield. Nutrient formulations included BBM, modified BBM with elevated nitrogen (double nitrate concentration), and commercial algal growth media for comparative analysis. Triplicate experiments were performed for each condition.

Cell density was monitored daily via direct counting (hemocytometer), optical density (OD_{680}), and dry weight (mg/L) after centrifugation and oven-drying (105°C for 24 h). Chlorophyll a content was quantified spectrophotometrically using 90% acetone extraction. Reproductive stages (autosporulation, daughter cell release) were observed using a compound microscope equipped with a digital camera, and cell division rates were calculated using standard doubling time formulas.

Biomass Harvesting and Preparation for Aquaculture

Once stationary phase was reached (~7 days), *Ch. vulgaris* biomass was harvested via low-speed centrifugation (3500 rpm, 10 min) followed by triple washing with sterile distilled water. The biomass was either used fresh (wet paste) or freeze-dried for inclusion in fish feed formulations. Proximate composition of the harvested biomass was analyzed using AOAC methods (2019) to determine crude protein, lipid, carbohydrate, ash, and moisture content.

Experimental Fish Farming System

To evaluate the application of *Ch. vulgaris* in aquaculture, a recirculating aquaculture system (RAS) was set up with juvenile Nile tilapia (*Oreochromis niloticus*), average weight 5.2 ± 0.3 g. The experiment consisted of four treatments (T1–T4) in triplicate:

T1 (Control): Commercial basal feed

T2: Basal feed + 5% *C. vulgaris*

T3: Basal feed + 10% *C. vulgaris*

T4: Basal feed + 5% *Ch. vulgaris* + live *Ch. vulgaris* addition in water (5×10^5 cells/mL)

Each tank (150 L capacity) housed 20 fish and was aerated continuously. Water temperature (28 ± 1°C), dissolved oxygen (>6 mg/L), pH (7.0–7.5), and ammonia/nitrite/nitrate levels were monitored using a multiparameter probe and standard colorimetric methods.

Growth Performance and Water Quality Assessment

Fish were fed twice daily (3% of body weight) for 45 days. Growth performance indicators including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate were recorded biweekly. Water quality parameters were monitored every three days to assess the biofiltration potential of *Ch. vulgaris* (especially in T4).

Statistical Analysis

All quantitative data were expressed as mean ± standard deviation. Statistical comparisons between treatments were conducted using one-way ANOVA followed by Tukey's HSD post hoc test at a significance level of $p < 0.05$. Growth trends and algal productivity across different experimental conditions were analyzed using regression analysis. Statistical analyses were performed using SPSS version 27.0 and OriginPro 2024.

3 Results and discussion

In the Bukhara district of the Bukhara region, there is an artificial reservoir LLC "BUKHOROBALIQ," in which there are all the conditions for organizing fishing. The fishery ponds are located 20-22 km south of the center of the city of Bukhara, with a total area of 293-305 hectares. Of these, about 200 hectares are reservoirs for raising fish and fish fry. The Khyrabad Canal and the Central Bukhara Collector are the main sources of water for fishery ponds.

Scientific research was conducted in 2023-2024-2025. Preliminary studies began with the collection of water and phytoplankton samples from the ponds of the fish farm. Water and phytoplankton samples were collected all seasons.

No later than 12 hours after the collection of water samples, at the Research Laboratory "Biotechnology and Ichthyology" under the Department of "Biotechnology and Food Safety" of the Faculty of Natural Sciences and Agrobiotechnology of Bukhara State University, Yu.Yu. Hydrochemical analyses of water samples taken from ponds were carried out using Lure methods.

Taxonomic determinants of many scientists were used in the study and systematic analysis of phytoplankton of fish ponds of "BUKHOROBALIQ" LLC.

In 2023-2024-2025, research work was carried out to study the species composition of microscopic lower algae in fish ponds of "BUKHOROBALIQ" LLC. During the conducted research, the species composition of green algae found in the composition of microscopic lower organisms of fish ponds, their distribution by seasons, and green biomass were analyzed.

The total area of Bukharabaliq LLC's fish ponds is approximately 293-305 hectares. Its fishery ponds have been organized, that is, the water-covered part covers 200 hectares. Samples of phytoplankton were collected from fish ponds using the Apstein net. The nylon mesh was of the brand No 55-76, and the diameter of the water inlet ring was 20 cm.

The number and quantity of phytoplankton were determined using a specially prepared glass container (Batometer). During the collection and fixation of phytoplankton samples, samples collected from various biological points of fish ponds at a depth of 0.5-1.0 m were used.

Collection and analysis of samples were carried out according to generally accepted methods. The collected samples of phytoplankton were treated with 3-4 drops of a 4.0% formalin solution and kept in a dark, dry place for one day, and the species composition was analyzed.

The number and composition of species of microscopic lower algae, their morphophysiological characteristics were studied and fully studied under a B-380 light microscope. The quantity of phytoplankton organisms in fish pond water was determined in ex/l, and the biomass in mg/m3 and g/m3.

The methods and scientific research of the following scientists were used to isolate pure species of microalgae from fish ponds of the limited liability company "Bukharabaliq" and to use their biomass in various sectors of the national economy.

In addition, using the scientific works of N.P. Gorbunova in 1991 and G.K. Plotnikov in 2017, the number and green biomass of microscopic algae were determined.

As a result of the conducted research, it was established that the systematics of phytoplankton species in the fish ponds of Bukharabaliq LLC is practically the same in all fish ponds, however, in some fish ponds they systematically differ slightly.

In the fish ponds of Bukharabaliq LLC, microscopic lower algae grow and develop throughout all stages of vegetation. The intensity of growth and development of microscopic lower algae also varies depending on the temperature of the fish pond water.

Microscopic tuban algae in fish ponds most often produce a very large amount of green phytobiomass from mid-spring (April 15) to early summer (June). During this period, the color of the water in fish ponds also changes. From the last ten days of April to mid-October, microscopic lower algae grow and develop rapidly. At the end of April and May, when the climatic conditions of the Bukhara region change and the air temperature is 28-30°C, an optimal environment for the growth and development of microscopic lower algae appears, and they actively reproduce and develop.

When the water temperature in fishery ponds reaches 22-24°C, that is, from the first ten days of June to the second ten days of August, the growth and development of microscopic lower organisms occurs actively.

In the last ten days of November, in the last month of autumn, due to a sharp decrease in air temperature, the water temperature in fishery ponds also decreases significantly. As a result, the developmental stage of most microscopic lower green algae stops. At the same time, fish from ponds specializing in commercial fish farming are caught, and the water in the ponds is completely drained and dried.

Fish ponds of Bukharabaliq LLC contain a large amount of biogenic minerals in the waters of the Central Collector and canals. This leads to the active reproduction of phytoplankton organisms.

The quantity and green biomass of microscopic lower algae in fish ponds change throughout the season and year. The reproduction of microscopic lower algae and the distribution of green mass in fishery ponds exhibits biodiversity due to a number of factors. One of the first is associated with the water of the reservoir and its mineral composition, that is, the amount of biogenic minerals.

The "Bukharabaliq" LLC fish farm, located in the Bukhara district of the Bukhara region, is one of the largest artificial fish farms not only among the districts, but also in the Bukhara region, which has been engaged in fish farming for many years. The total area of the fish farm reaches 293-305 hectares. The farm has a total of 39 ponds, the smallest of which occupies an area from 2.0 hectares to the largest - 55 hectares.

For many years, herbivorous and plankton-eating fish of various breeds have been kept in these ponds. At the bottom of the ponds, during the growing season, there is a high layer of mud and clay, the depth of which is 1.0-1.5 meters, and in some cases not less than 2.0

meters. At the bottom of some fish ponds and near the shores of others, one can find various filamentous and branched aquatic plants, as well as tall reeds, reeds, and loach.

The transparency of the water in the ponds varies depending on the season, and when checked, it was determined that it is 45-50 centimeters, and in shallow-water fish ponds, it is clear to the bottom (when checked using a Secchi disc). It was observed that the group indicators (pH) of water (H+) or (OH-) are in the range of 6.0-8.0. The sources of water supply for these fishery ponds, as mentioned above, are the Khyrabad Canal and the Central Bukhara Collector.

Based on the results of the analysis of algo-hydrobiological scientific research carried out in 2023-2025 in the fishery ponds of BUKHOROBALIQ LLC, it became known that a total of 106 species of microscopic lower algae are found in the fishery ponds of this area. Samples from 5 divisions, 9 genera, 23 tribes, 44 families, 69 genera are presented in Table 1.

Table 1. Taxonomic composition of the algoflora of the ponds of Bukharabaliq LLC fish farms.

Sections of algae	Class (ancestor)	Order (tribe)	Family	Genus	Species
Cyanophyta	1	3	13	18	20
Euglenophyta	1	1	2	5	13
Dinophyta	1	2	2	3	3
Bacillariophyta	2	7	10	20	27
Chlorophyta	4	10	17	23	43
Jami:	**9**	**23**	**44**	**69**	**106**

According to the data presented in the table above, in the fishery ponds of Bukharabalik LLC, where the research work was carried out, the green algae (Chlorophyta) division led in the number of species, which amounted to 40.5% (43 species) of the total number of species. As can be seen from the table above, the following places are occupied by the divisions Bacillariophyta 25.4% (27 species), Cyanophyta 18.8% (20 species), Euglenophyta 12.2% (13 species), Dinophyta 2.8% (3 species).

In fish ponds located on this area of the fish farm, it was established that Euglena (4 species), Nitzschia (3 species), Merismopedia (3 species), Ulotrhix (3 species), Spirogyra (3 species), Phacus (3 species), Tetrahedron (2 species), Closterium (2 species) lead in the number of species.

In the ponds of the Bukharabaliq LLC fish farm, 23 species of microscopic lower algae are included in the 8 orders of algae listed in the table above, accounting for 21.7% of the total number of microalgae.

In spring, in the reservoirs of the Bukharabaliq LLC fishery farm, the presence of such low-order microscopic aquatic plants as Volvox ehrenberg, Peridiniopsis quadridens, Euglena gracilis, Cyclotella meneghiniana, Discostella stelligera, Odontidium hyemale, E. salina, Chlamydomonas reinhardtii, Spirocoleus fragilis, Leibleinia epiphytica, E. oblonga, Calothrix brevissima, Willea apiculata was observed.

By summer, as a result of a slight increase in the water temperature of fishery ponds and the oxidation of minerals in the water of fishery ponds, as well as their decomposition by reducing agents, most of the above-mentioned plant species continue to grow. Along with the listed plants, the active growth and development of such species as Oscillatoria planctonica, M. tenuissima, Ulothrix tenerrima, M. Elegans Fragilaria amphicephaloides, Spirulina princeps, U. Limnetica, Microcystis protocystis, F. capucina, Merismopedia punctata, which grow in colonies and have branched thallus, were observed many times in fishery ponds.

In some ponds of shallow fish farms, the phenomenon of "flowering" of the vegetative layer of water, "blossom," is often observed.

The water from the Bukharabaliq LLC fishery ponds is not discharged to the surrounding areas, but is partially discharged into the Central Collector, located not far from the farm (only during the fishing season). The decrease in water in fishery ponds occurs due to the evaporation of water from the surface of fishery ponds and the absorption of water into the ground.

The Bukhara Central Collector, completely bounded by the southwest of Bukharabaliq LLC, is formed by groundwater and wastewater from agricultural crops. The average depth of this collector is 1.5-2.5 m. Like all collectors, the bottom of these collectors consists of mud and clay. With rising air temperatures, most of the collector area is covered with branched filamentous and higher aquatic plants. Similar to fishery ponds, areas in the inner part of the collector, not far from the shore, are covered with tall vegetation, such as reeds and cattails.

According to the results of algo-hydrobiological studies conducted in the Bukhara Central Collector, which borders the southwestern part of Bukharabaliq LLC, 116 species of microscopic lower algae were found in these collectors. Their belonging to 75 genera, 42 families, 26 orders (tribes), 11 classes (ancestors) was analyzed and presented in Table 2.

Table 2. Taxonomic composition of the algoflora of the Bukhara Central Collector.

Algae	Class (ancestor)	Order (tribe)	Family	Genus	Species
Cyanophyta	1	4	10	13	**15**
Bacillariophyta	3	7	13	25	**37**
Dinophyta	1	2	2	5	**6**
Euglenophyta	1	1	2	5	**13**
Chlorophyta	5	12	15	27	**41**
Jami:	**11**	**26**	**42**	**75**	**112**

From the data presented in Table 2, it is clear that in relation to the total number of species, the divisions Dinophyta - 6 species (5.3%), Euglenophyta - 13 species (11.6%), Chlorophyta - 41 species (36.6%), Bacillariophyta - 37 species (33.03%) dominate with a high number of species in the waters of fishery ponds. 15 species (13.4%), Cyanophyta ranks among the divisions before the divisions Dinophyta and Euglenophyta.

According to the results of the algo-hydrobiological analysis of fishery ponds, the number of species of such genera as Nitzschia (6), Cosmarium (5), Phacus (4), Euglena (3), Merismopedia (3), Spirogyra (2) prevails in these ponds. These 5 genera include 23 species, which make up 21.7% of the microalpha flora of ponds located in the above-mentioned territory.

It was noted that the average temperature of the water in the fishery ponds of "BUKHOROBALIQ" LLC in the spring period is 15-16°C, and in the summer period it is 22-24°C. During seasonal analysis of the amount of mineral salts in the water of fishery ponds during the winter, spring, autumn, and summer seasons, their mineral indicators changed on average to 2215-2403 mg/l. In most fishery ponds, the hydrogen indicator (pH) of water fluctuates within the range of 6.5-8.5, and the turbidity of water fluctuates within the range of 0.50-0.65 m during the seasons.

In spring, during the analysis, such species as Volvox ehrenberg, Leibleinia epiphytica, Chlamydomonas reinhardtii, Odontidium hyemale, Willea apiculata, Euglena salina, Cyclotella meneghiniana, Calothrix brevissima, Spirocoleus fragilis, Euglena oblonga, Peridiniopsis quadridens, Euglena gracilis, Discostella stelligera were observed, and in summer, such species as Spirulina princeps, Fragilaria amphicephaloides, Merismopedia elegans, Merismopedia tenuissima, Fragilaria capucina, Ulothrix tenerrima, Oscillatoria planctonica, Merismopedia punctata, Ulothrix limnetica, Microcystis protocystis rapidly multiplied, grew, and developed in pond waters that receive water from slightly saline collector waters. Despite the decrease in water and ambient temperature in fishery ponds in autumn, diatoms, dinosaurs, and euglena algae were found in relatively large quantities in pond water samples.

During the analysis, it was noted that the algoflora of the area of the ponds of the fishery of LLC "BUKHOROBALIQ" can vary depending on the water source of the ponds. The ponds, located to the west of the fish farm, are supplied with water from the Khyrabad Canal, therefore they are filled with muddy, fresh water. Water from the Central Bukhara collector, rich in organic and mineral substances, is also partially supplied to the fish ponds in the east of the farm. Therefore, during the research, it was proven that water saturation sources have a great influence on the formation of the algoflora of fish ponds located in this area.

4 Conclusion

During the studies conducted in fish ponds, a total of 106 species of microalgae were identified, of which Chlorella vulgaris, belonging to the Chlorophyta division, was isolated in laboratory conditions as a monoculture and successfully propagated. Optimized conditions—temperature 25°C, pH 7.5, light intensity 150 μmol photons m−2 s−1, and a nitrogen-rich environment—were determined to be the most favorable factors for the formation of high biomass of Ch. vulgaris. When the obtained biomass was tested in fish feed, positive results were noted in terms of growth rates, productivity, and survival rates. Option T4 (main feed + 5% Ch. vulgaris + addition of live Ch. vulgaris to water, 5 × 105 cells/ml) showed the greatest effectiveness. In addition, no increase in the amount of ammonia, nitrates, and nitrites in the aquatic environment was observed, confirming that the use of microalgae does not have a negative effect on fish growth rates. It has been proven that Chlorella vulgaris can be effectively used not only as a feed supplement with high nutritional value, but also as a bioagent that ensures environmental sustainability in aquaculture.

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