Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(3): 955 – 966 (2023)
www.ejabf.journals.ekb.eg

Use of Microalgae in the Production of Feed for Aquaculture

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ARTICLE INFO
Article History:
Received: June 9, 2022
Accepted: Feb. 19, 2023
Online: June 30, 2023

Keywords:
Chlorella,
Scenedesmus,
Ankistrodesmus,
Chlorococcum,
Microalgae,
Fish feed,
Aquaculture

ABSTRACT

This paper analyzed the dry mass output of microalgae from wet biomass in terms of their genus. The amount of dry mass outputs of microalgae was determined as follows: 27.6% in *Ankistrodesmus* strains, 25.2% in *Scenedesmus* strains, 23.4% in *Botryococcus* strains, 26.2% in *Chlorella* strains and 27.5% in *Chlorococcum* strains. Protein and fat content of microalgae were observed to differ sharply from each other when grown in Chu-13 nutrient medium. At the same time, it was noted that microalgae of the genus *Ankistrodesmus* contain 43.2-46.4% protein and 27.4-32.2% fat. In the same nutrient medium, microalgae of the species *Scenedesmus* retain 48.8-52.8% protein and 27.4-28.6% fat, respectively. Representatives of the species *Botryococcus* retain relatively less protein (46.2–46.8%) and fat (26.2–26.4%). While representatives of the species *Chlorococcum* and *Chlorella* retain high protein (46.4–48.8%) but low-fat synthesis (15.6–18.4%).

INTRODUCTION

The bulk of feed products produced for agriculture (livestock, poultry, fisheries) around the world at the end of 2016 exceeded 1 billion tons **(Alltech Global Feed Survey, 2017)**. Only 4%, or 40 million tons, is (are) intended for aquaculture **(Feed International's World Feed Panorama, 2016)**. Southeast Asian countries (primarily China) are the absolute leaders in the feed production for aquaculture, and their products account for almost half of the total world production. In particular, Latin America produces an average of 2.88 million tons of feed per year (2.3 million tons for fish; 0.57 million tons for shrimp), the European countries produce 2.0 million tons, North America- 2 million tons, Africa (mainly Egypt - 0.75 million tons and Nigeria) produces around 1 million tons of feed products **(Alltech Global Feed Survey, 2017)**.

China is the world leader in the production of feed for aquaculture, producing 17.30 million tons of feed a year, accounting for 40% of the world's production. At the same time, China's aquaculture industry accounts for 3/4 of the world's feed production. Apart from China, Vietnam (2.80 million tons/year), Norway (1.79 million tons/year), Chile (1.24 million tons/year) and Indonesia (1.23 million tons/year), India (1.16 million tons/year), USA (1.00 million tons/year) are the largest producers of feed products **(Alltech, 2016)**. The development of this industry can be explained by the specialization of local production. China produces a very large amount of feed for carp (almost 62% of the feed intended for aquaculture), while India, Thailand and Indonesia grow large amounts of feed for shrimp (66%; 42%; 62%, respectively). Large quantities of food products are produced in the United States (40%), Vietnam (36%) and Bangladesh (35%) for trout, while in Peru they are produced for trout (74%); however, Norway (94%), Canada (86%) and Chile (85%) feed products are produced primarily for salmon **(Alltech, 2016)**. Remarkably, China cannot be a leader in the production of feed products for all areas of aquaculture. In particular, 75-80% of the world's production of salmon and trout is accounted for by Chile and Norway. In the Russian Federation, the total demand for fisheries is 250,000 tons, but an average of 100 ton of feed is produced per year, and the rest is met mainly by imports **(Feed International's World Feed Panorama, 2016)**.

Outside China, there are four major manufacturers that supply aquaculture feed, accounting for 35% of world production. These are the following companies: Cargill (USA) merged with Ewos (Norway) in 2015, BioMar (Denmark), Nutreco (Netherlands), and Biomin (Austria). In addition, companies such Alltech Inc., Aller Aqua, Avanti Feeds Ltd., Beneo, Cermaq ASA, Charoen Pokphand Foods Company Ltd., Dibaq Aquaculture, Guangdong Evergreen Feed Industry Co., Ltd., NK Ingredients Pte Ltd., Norel Animal Nutrition, Nutriad, Nutreco N. V., and Tongwei, Ridley Aqua-Feed play an important role in supplying aquaculture food products. The state of Peru controls 40% of the world's manufacturing industry in the production of fish meal, which is the main component of traditional feed products for aquaculture. The world feed market for aquaculture is estimated at $ 57.7 billion (2012), with an annual growth rate of 11.4% projected to reach $ 122.6 billion by 2019. However, world production did not reach this figure. Demand for products in the world market for aquaculture facilities also varies. In particular, feed products are sold on the world market for carp (40%), tilapia (20%), crustaceans and salmon (about 20%) in addition to mollusks, crucian carp and others **(Feed International's World Feed Panorama, 2016)**. By 2021, feed products for carp and crustaceans will dominate the global market, according to experts from the research organization Transparency Market Research. Feed production for mollusks and salmon is also expected to grow sharply. In addition, according to its geographical location, the Asia-Pacific region is expected to control 65% of the world market and the rapid development of aquaculture in them. In addition, Europe may rise to the second place in terms of feed production for aquaculture **(Aqua Feed Market, 2013)**.

According to experts, radical changes are expected to take place in the feed industry for aquaculture. There are many factors of drastic change, which can be summarized as follows: aquaculture feed (soy, wheat and corn, fish-based feed), traditional technology based on the trend of "fish-to-fish" in production, as well as its current state. These factors do not meet the requirements of long-term sustainable development of the world fishing industry **(Khujamshukurov & Nurmuxamedova, 2016)**.

MATERIALS AND METHODS

Study samples

The following genera of microalgae were isolated and algologically purified from different regions of Uzbekistan. *Ankistrodesmus* (*Ankistrodesmus* sp. 15, *Ankistrodesmus* sp. 20, *Botryococcus* (*Botryococcus* sp. 5, *Botryococcus* sp. 14, *Scenedesmus* (*Scenedesmus* sp.1, *Scenedesmus* sp.7, *Chlorococcum* (*Chlorococcum* sp.3, *Chlorococcum* sp.4, and *Chlorella* (*Chlorella* sp.3, *Chlorella* sp.4). A Chu-13 nutrient medium (g/l) was used for growing algae: KNO_3-0,2, K_2HPO_4 - 0,04, $MgSO_4 \times 7H_2O$ -0,1, $CaCl_2 \times 6H_2O$ -0,08, iron citrate- 0,01, citric acid – 0.1, boron - 0,5 ppm, $MnSO_4 \times 7H_2O$ - 0,5 ppm, $CuSO_4 \times 5H_2O$ - 0,02 ppm, $CoCl_2 \times 2H_2O$ -0,02 ppm, $Na_2MoO_4 \times 2H_2O$-0,02 ppm, pH 7,5 **(Safarov *et al.*, 2019)**.

Cultivation of algae

In the cultivation of algae, CO_2, light and air were given as standard **(Mata *et al*., 2010)**. Temperature was maintained from 27- 30°C. The selected microalgae were cultivated in a bioreactor for the cultivation of microalgae in closed conditions (Fig. 1), as well as in ponds for the cultivation of microalgae of open and spiral type (Fig. 2) for high-volume production.

Fig.1. Bioreactors for the cultivation of microalgae in closed controlled conditions

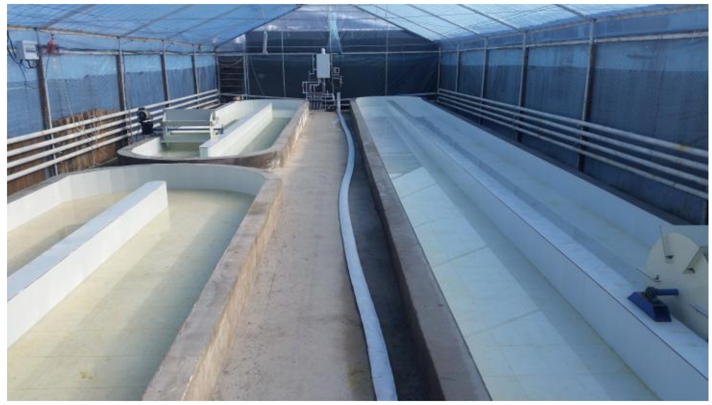

Fig. 2. Microalgae cultivation ponds of open and spiral type

Methods used in the study

Standard methods were used to determine the microscopy of microvilli and their morpho-cultural properties **(Shakirov *et al*., 2014)**. The number of cells was calculated in Goryaeva's chamber using standard methods. The Loury method **(Khujamshukurov, 2007)**, and the standard method for determining the amount of fat were used to determine the amount of protein in the study samples **(Rajasri *et al*., 2012)**.

Statistical analysis

The calculation of statistical error, mean, reliability intervals and standard deviations to the experimental data was performed using the computer program STATISTICA 6.0 and standard methods. The statistical significance of the results was determined using the Student t-criterion.

RESULTS

The total biomass and dry biomass production of all microalgae under study were determined during the three-day growth phase of culture development.

According to the results obtained, it was noted that the strain *Ankistrodesmus* sp.15 produced 12.62% of wet biomass, from which an average of 3.35% of dry matter was recorded. The strain *Ankistrodesmus* sp.20 produced 11.36g/ l of biomass, from which 3.24 g/l of dry biomass was extracted, accounting for 27.5% of the total mass of the cell (Fig. 3). While, the strains *Botryococcus* sp.*5*. and *Botryococcus* sp. 4 of *Botryococcus* genus produced 9.22 & 8.62g/ l of wet biomass, respectively, and 2.12 & 2.04g/ l of dry biomass was recorded, respectively (Fig. 5). Dry matter emissions from total wet biomass averaged with a value of 23.4%. The strains *Scenedesmus* sp.7 and *Scenedesmus* sp.1 of the *Scenedesmus* genus produced wet biomass of 11.44 & 10.66g/ l, respectively, while dry biomass yielded 3.08 & 2.48 g/l, respectively (Fig. 4). Dry matter excretion from total cell biomass recorded an average of 25.16%.

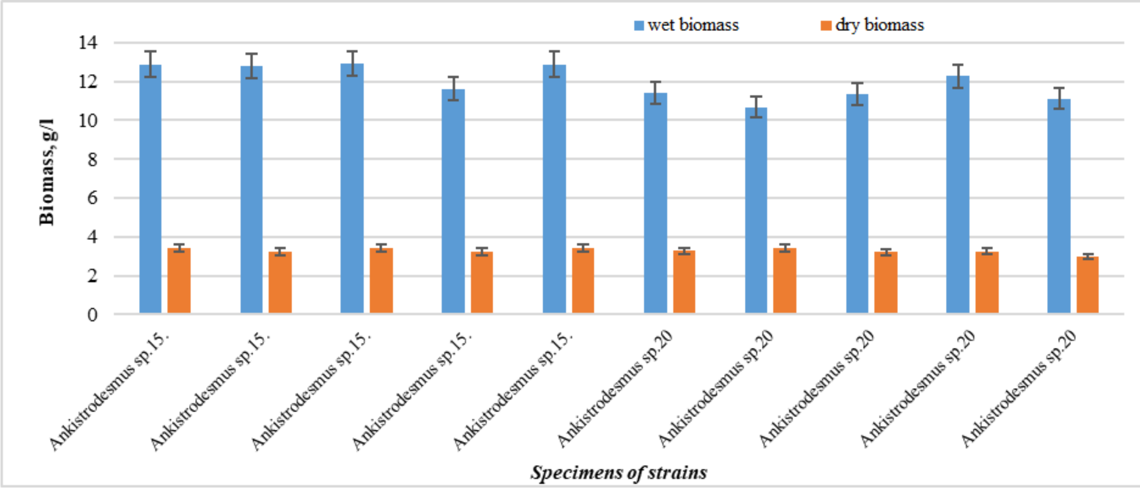

Fig. 3. Biomass production of species *Ankistrodesmus strains*; n=3.

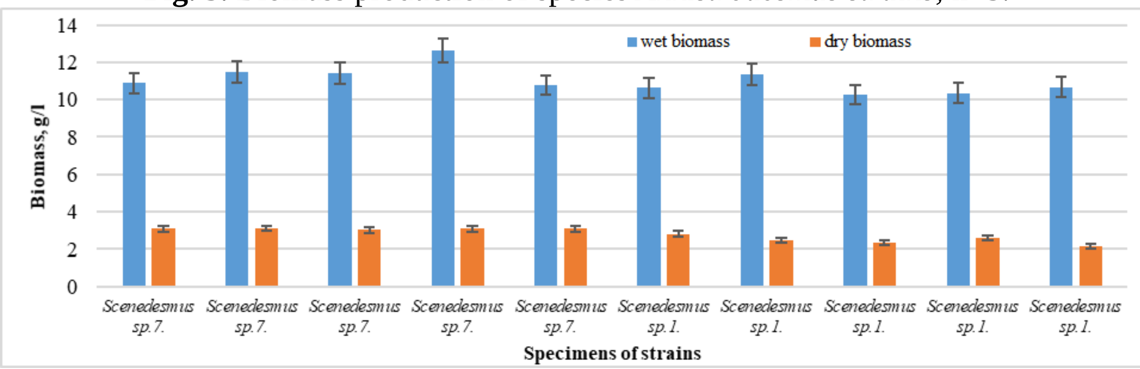

Fig. 4. Biomass production of *species Scenedesmus*; n=3.

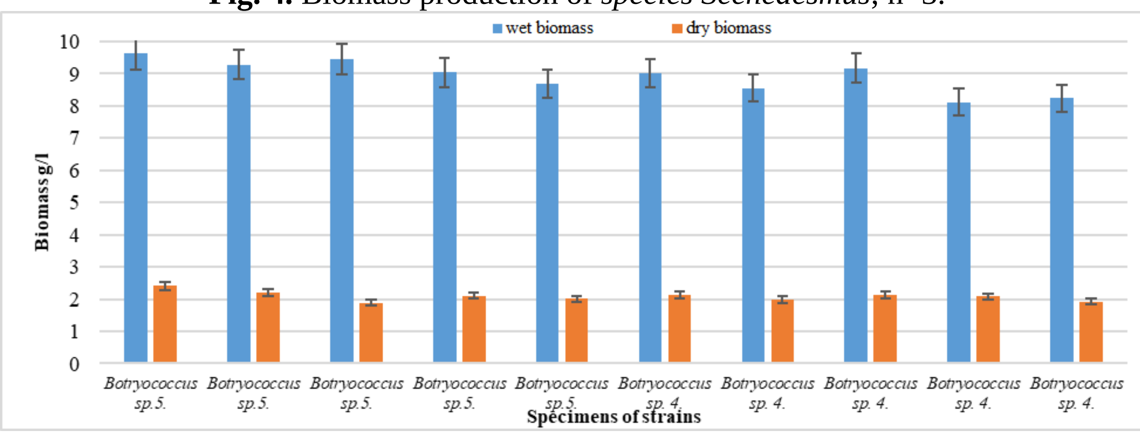

Fig. 5. Biomass production of species *Botryococcus*; n=3.

While the strain *Chlorella* sp.3 of *Chlorella* genus produced 10.02 g/l wet biomass, the strain *Chlorella* sp.4. was noted to produce 9.22g/ l of wet biomass (Fig. 6). The dry mass yield from these strains was 2.56- 2.48g/ l, respectively. It was noted that the dry mass yield from total wet biomass was 26.2%.

The strain *Chlorococcum* sp.4 of *Chlorococcum* genus produced 12.68 g/l of wet biomass, of which 3.64% g/l of dry biomass was detected (Fig. 7). In the strain *Chlorococcum* sp.3 of this genus, the wet biomass yield was 11.48 g/l, of which 3.01 g/l was recorded. Of the total biomass, the dry biomass yield was 27.5%.

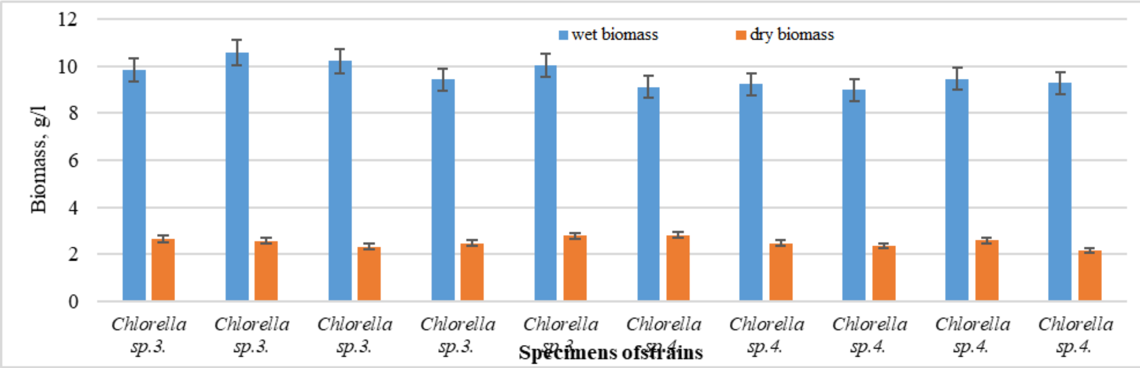

Fig. 6. Biomass production of species *Chlorella*; n=3.

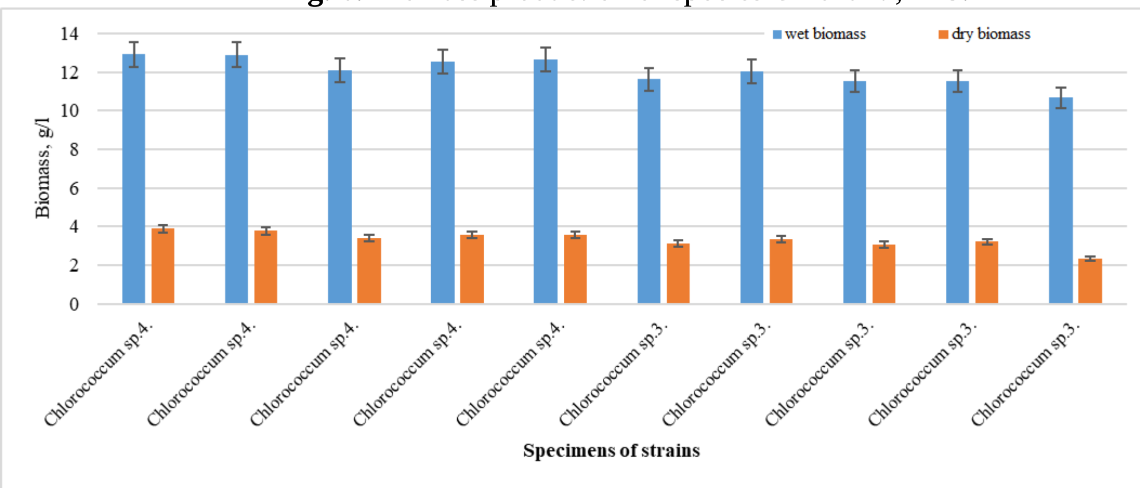

Fig. 7. Biomass production of species *Chlorococcum*; n=3.

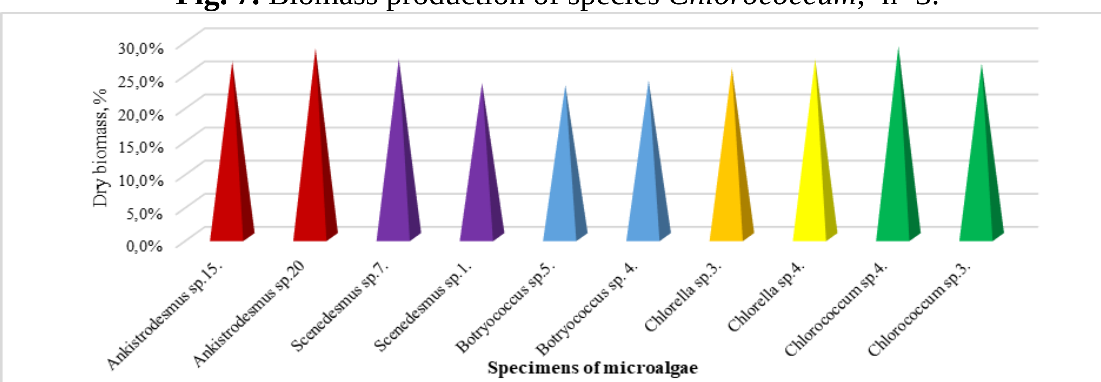

Fig. 8. Output of dry biomass from wet biomass according to microalgae genera, (%) n=3

It is known that the process of obtaining dry biomass from wet biomass in the production process determines industrial productivity and economic efficiency. During the study, the dry biomass output of wet algae from wet biomass was analyzed according to genera, and it was determined that the amount of dry biomass was 27.6% from *Ankistrodesmus*, 25.2% from *Scenedesmus*, 23.4% from *Botryococcus*, 26.2% from *Chlorella* and 27.5% from *Chlorococcum* (Fig. 7). In a subsequent research, the protein and fat storage of micronutrients selected as an object were addressed (Fig. 8).

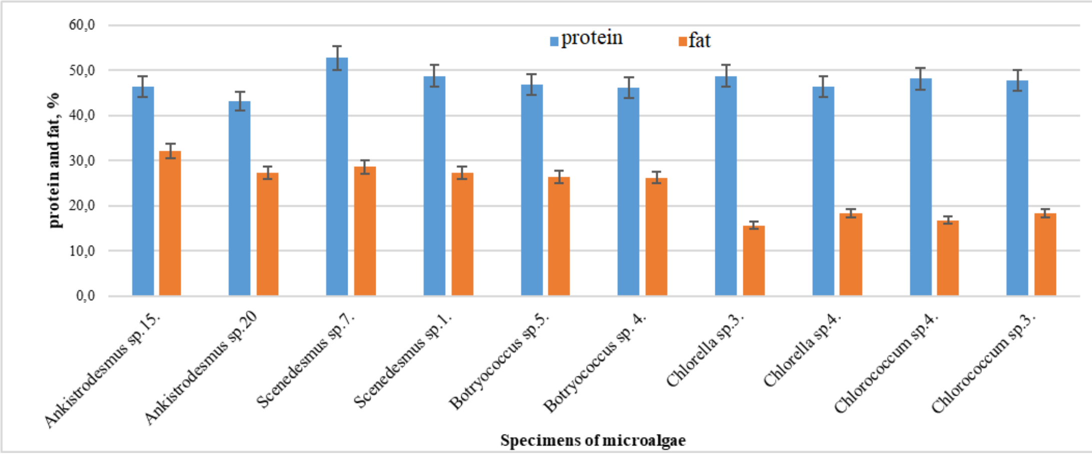

Fig. 9. Protein and fat production of microalgae genera (% of dry matter); n = 3

Data in Fig. (9) show that the protein and fat storage of micronutrients differ dramatically from each other in the Chu-13 nutrient medium. Microalgae belonging to the genus *Ankistrodesmus* contains 43.2- 46.4% protein and 27.4-32.2% fat (*Ankistrodesmus* sp.20; *Ankistrodesmus* sp.15). In the same nutrient medium, micronutrients belonging to the genus *Scenedesmus* can be seen to store up to 48.8-52.8% protein and 27.4-28.6% fat, respectively (*Scenedesmus* sp.7 and *Scenedesmus* sp.1).

According to the results of the study, representatives of the genus *Botryococcus* were recorded as a microalgae that retained relatively less protein (46.2-46.8%) and fat (26.2-26.4%). The members of the *Chlorococcum* and *Chlorella* genera exhibited a high protein storage (46.4–48.8%) and a very low fat storage (15.6–18.4%), compared to all the algae genera studied.

DISCUSSION

According to literature, there are many species of *Chlorella*, *Scenedesmus*, *Ankistrodesmus* and *Chlorococcum*, belonging to the genus *Chlorococcus* according to their systematic status. A percentage of 18.6% lipids occurred in the 4-week culture of *Ankistrodesmus brounii* strain, whereas 33.7% lipids occurred in the 7-week culture, which is consisted of 61.6% - saturated fats and 6.8% - glycerol. Fatty acids consisted of 13% - linolin, 54% - olein, 33% - polmitin and furthermore, additional caproic, lauric, polmitoleic acids are found **(Zeng *et al.*, 2009)**. Depending on the type of microalgae, it produces the following amount of lipids: *Scenedesmus dimorphus* contains 14-16%, *Prymnesium parvum*-22-38%, *Euglena gracilis* -14-20 %, *Chlorella vulgaris* -14-22%, *Dunaliella salina* -16-44%, *Haematococcus pluvialis* -25-45%, *Tetraselmis suecica* - 20-30%, *Isochrisis galbana* - 22-38%, *Nannochloropsis* sp. -33-38%, *Stichococcus sp* -40-59% and *Botryococcus brounii* -80%. According to Y. Chisti, microalgae produce 10-100 times more fat than higher algae **(Barghbani *et al.*, 2012)**. Microalgae can be found in different regions of the globe, in oceans and seas, in warm water basins, glaciers, even in the atmosphere, and until now scientists have noted the existence of 35-40 thousand different species **(Becker, 2004)**.

1. It is known that microalgae are widely spread organisms found in all water bodies in the globe; there are more than 50,000 species of them, and only about 30,000 of

them are very ancient organisms that have been studied **(Cho *et al.*, 2011)**. A.M.Muzaffarov found that there are about 2965 types of microalgae in natural water bodies in Central Asia **(Minyuk, 2008).**

2. It has been noted that algae such as chlorella, scenedesmus, and ankistrodesmus can produce 50-60 tons of dry biomass per hectare of water basin when grown in favorable conditions. In addition, algae prevent the increase of carbon dioxide gas in the atmosphere. For example, algae in the ocean absorb 2 gigatons of CO_2 per year, compared to 1.5 gigatons for land plants. Microalgae are an important carbon reservoir of the biosphere **(Minyuk *et al.*, 2008)**. During the initial industrial cultivation of microalgae, *Chlorella* and *Scenedesmus* were given actual importance **(Lambertus *et al.*, 2018)**.

3. In the 20th century, the study of microalgae developed rapidly during the past 50 years in order to produce practical and economically important products from various microalgae; a large-scale research work on *Chlorella Nihon* culture has started in Japan **(Abd-El-Monem *et al.*, 1998)**.

Microalgae have attracted the attention of many companies and researchers due to the following facts: the cultivation of microalgae requires less labor compared to agricultural crops; they do not pollute the environment; the oils obtained from them are not used as food products; in addition, they can compete with natural diesel in the production of biodiesel and finally being economically effective **(Abd-El-Monem *et al.*, 1998)**.

In Uzbekistan since 1957, under the leadership of A.M.Muzaffarov, academicians of the Academy of Sciences of Uzbekistan, and a group of scientists studied the effect of using microalgae in various fields of the economy and its biological properties. Although significant data have been reported on the use of *chlorella* in animal husbandry, further research is needed on the wider use of algae in the future. Currently, microalgae are widely used in poultry and livestock feeding, sewage treatment, air regeneration (purification) in space flights and in fertilizers. In the near future, the widespread use of unicellular algae in other areas of the national economy is not far off.

In addition, work is being carried out to extract protein, vitamins and other products from microalgae. In the cultivation of microalgae, it is important to pay particular attention to the average temperature of the year and the long duration of sunny days. Long duration of sunny days in Uzbekistan is a favorable condition for growing microalgae.

The morpho-physiological and chemical properties of microalgae undergo changes to a certain extent based on the temperature of the area where they live, pH environment, biotic, abiotic, anthropogenic influences.

Currently, there are about 30,000 types of microalgae known to science, among which there are blue-green, green, yellow-green, brown, red, and golden algae. In addition to chlorophyll, the cells of these algae contain various pigments that determine their color. In addition to water, there are also species of algae that grow on wet soils, tree bark, ponds, and stones on the banks of the river **(Lombardi *et al.*, 2007)**.

These algae are divided into the following sections depending on the origin, photosynthesis apparatus (chromatophore or chloroplast), accumulation of photosynthesis products in the cell and the structure of mobile cells: blue-green algae-Cyanophyta, green algae-Chlorophyta, golden algae-Chrysophyta, diatom algae-Bacillariophyta, brown algae-Phaeophyta, pyrrophyte algae-Pyrrophyta, euglenophyte algae-Eyglenophyta and red algae-Rhodophyta algae **(Monteiro *et al.,* 2011)**.

Chlorococcum algae is one of the large sections of green algae, and as a result of studying their taxonomy, *Chlorococcum* (Chlorococcaceae) microalgae includes about 1200 species **(Etienne *et al.,* 2004)**. Their cells are spherical and ellipsoidal, rarely clustered. The size of the cells is 1.5-10 mkm. The cell membrane is usually thick, smooth, and not covered with a mucous substance. Chloroplasts are spherically centered, the perenoid is open, or covered with a thin starch skin, often thinly smooth. Typically, microalgae belonging to the genus *Chlorella* reproduce using 2-8 autospores. The mother cell divides and multiplies every 2- 4 hours **(Dayananda *et al.,* 2005)**.

Chlorococcum humicola and *Chlorococcum infusionum* cells are granular, sometimes elliptical to spherical. Reserve substances are starch and fat. Reproduction occurs with zoospores of equal length, binary, sometimes with aplanospores. Reproduction is carried out by sexual - isogamy. Zygospores have a smooth or patterned shell. Zoospores are released through the breakdown of the mucous membrane of the mother cell. They are widely distributed in nature **(Metzger & Largeau, 2005)**.

According to species diversity and interspecific taxa, this genus is the most numerous among chlorococcal algae. About 1000 different representatives have been identified in the flora of the world. The widespread species belonging to the genus *Scenedesmus* are: *S. obliquus, S. acutus* and *S. lefevrii*, etc. **(Tsarenko, 1990)**.

Colonies of Ankistrodesmaceae family, Ankistrodesmoideae subfamily, *Ankistrodesmus* genus are variously comb-shaped, often united with the central part, and are rarely unicellular; the surface part is surrounded by a mucous substance, sometimes it is not clearly visible. Cells are elongated, straight or twisted, lanceolate, needle-shaped, and cylindrical, with tips gradually tapering and lanceolate. The bark is smooth, thin and colorless. Reproduction is performed by 2-4-8-(16) autospores. They are arranged parallel to each other in the mother cell and come out as a result of the rupture of the center of the mother cell shell. Cell size: 22-62 mkm long, 1.2-4.3 mkm wide.

Ankistrodesmus genus can be divided into 10 species, depending on the nature of the formation of autospores, their location in the cell, life form, and the formation of a mucous substance. Widespread species are *A. fusiformis, A. faicatus* and *A. falcatus* **(Jeon & Hegewald 2006)**.

Colonies of microalgae belonging to the family Botyococcaceae and the genus *Botyococcus* are spherical to indistinctly scaly, divided into separate parts as they grow larger, connected to each other by mucilaginous bands, the cells of the periphery (on the surface) of the colony are located radially in a goblet slime formation; the entire length of the cell or ¼ of the length of the colony rises. The chloroplast is yellow-green cup-shaped, sometimes feathery, covering the apical part of the cell, it is difficult to distinguish the pyrenoid. 2-4-8-16 autospores, located in parallel in the mother cell, reproduce or reproduce by division. A typical representative of the Botryococcus genus is *B. braunii* **(Jung *et al.,* 2016)**. Colonies are spherical to indeterminate, multicellular, arranged radially in colonial mucilage with dense cells at the periphery of the colony, colorless yellow-brown. Colonies are up to 1 mm in diameter, and cells are 5.7 - 12 x (2.5) - 3 -7.5 mkm long. General distribution: Asia, Africa, Europe.

Microalgae *Chlorella, Chlorococcum, Scenedesmus, Botruococcus* are several times superior to agricultural plants in terms of biomass accumulation and oil production.

When microalgae are grown in open-type special breeders (bioreactors), 70 tons of biomass per year can be obtained from one hectare of land **(Bajhaiya *et al.*, 2010)**.

The single-celled algae *Botruococcus*, *Chlorococcum*, *Scenedesmus* and *Chlorella* species are rich in lipids and are suitable objects for the production of biodiesel fuel. The microalgae *Botruococcus braunii* specifically has the ability to produce up to 80% of oil, compared to dry biomass **(Kumar *et al.*, 2011)**.

CONCLUSION

It is known that microalgae have a radical importance almost in all sectors of economy. They serve as a productive source in the production of biodiesel, bioethanol, biogas, food and feed products, as well as in the production of various agricultural biopreparations. Therefore, the biomass formed in the extraction of micro-aquatic biomass and the release of dry mass from this biomass are important. Therefore, in this study, it was found that microswaters form biomass over the generations and dry mass emerge from this biomass. Additionally, the dependence of protein and fat storage was addressed, which determines the nutritional value of microalgae in their genera. Notably, the protein and fat storage of micronutrients in the Chu-13 nutrient medium differs markedly from one to the other. It was noted that, microalgae of the genus *Ankistrodesmus* contain 43.2- 46.4% protein and 27.4- 32.2% fat. In the same nutrient medium, species *Scenedesmus* was observed storing up to 48.8- 52.8% protein and 27.4-28.6% fat, respectively. According to the results obtained, it is advisable to use strains of the species *Botryococcus and Chlorococcum* to obtain a feed with a complete nutrient content for the aquaculture industry.

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