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Isolation and identification of lipid-producing microalgae of Uzbekistan

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ABSTRACT

From water samples of the Syr-Darya and Amu-Darya are isolated 15 local microalgae belonging to the genus *Chlorococcum* and 13 to the genus *Scenedesmus*. Air-dry biomass of microalgae ranged from 1.29 to 2.79 g/l of environment. The lipid content of effective strains of *Chlorococcum* sp.4, *Chlorococcum* sp.8, *Chlorococcum* sp.12 and *Chlorococcum* sp.37 was 41.8 - 58.0% of the total dry biomass of microalgae. High lipid accumulation observed in cells of cultures *Scenedesmus* sp.29 and *Scenedesmus* sp.37- 40,3% and 48,0%, respectively.

KEYWORDS

Microalgae;
Chlorococcum;
Scenedesmus;
Lipid.

INTRODUCTION

Biodiesel is a substitute of diesel fuel, which is produced at the present time mainly from vegetable or animal oil[7].

Even by the most optimistic forecasts biodiesel from vegetable oil can not replace even a small fraction of diesel fuel in the future. Fortunately, it is possible to produce biodiesel from oil derived from microalgae. Many species of algae can accumulate the required amount of oil, which is enough for biodiesel[13]. Oil production in many species of algae exceeds oil production of the best cultured plants. Oil content in some algae may exceed 80% by dry weight of the biomass, usually microalgae contains oils 20-30%[6,13,17]. Algae use oil as reserving material. Oil from algae can be converted into biodiesel through the transesterification reaction, a simple chemical reaction with the alcohol (methanol or ethanol) which is catalysed by acids or alkalis. Biodiesel produced from vegetable oils (cotton, rapeseed, sunflower, soybean, palm, coconut, etc.) is now regarded as a viable alternative to dwindling oil reserves. However, in terms of population growth in the world manufacturing biodiesel from vegetable oil will create a serious shortage of foodstuffs and threatens significant damage to the environment[8,19]. Obtaining of biodiesel from algae lipids may be the only way to produce enough fuel to replace the current dependence on oil. Algae produces 7-31 times more oil than palm oil[12]. The best algae for biodiesel is unicellular green algae (microalgae). The content of lipids and oils from microalgae sometimes exceeds 70% by weight of dry biomass[18]. The presence of lipids, hydrocarbons and other complex oils depends on the algal species.

The purpose of this paper is to search and screening of local strains of microalgae, which are high productive on the accumulation of lipids.

MATERIALS AND METHODS

Obtaining of accumulative cultures of microalgae

For obtaining of accumulative cultures of unicellular green algae water samples were collected from the Syr-Darya and Amu-Darya in Uzbekistan.

For every 100 ml of aqueous sample was added to 200 ml of sterile medium of the modified "Chu-13"[16]. Inoculated environment with aqueous sample was incubated at 26°C-28°C in the light 150 μmol photons $m^{-2} \cdot s^{-1}$ in 30-45 days.

Isolation of microalgae

To isolate microalgae from accumulative cultures, samples were sown to 2% agar environment "Chu-13" using the dilution method and samples were placed on a light for the formation of colonies of microalgae. In the first week of cultivation, on the surface of the agar medium very small, separata colonies of microalgae appeared, and after three weeks colony size reached up to 1-3 mm. Each colony of microalgae was sowed on 5 ml medium "Chu-13" and the cultivation was carried out within 7-15 days, depending on the rate of growth of crops. Algological and bacteriological purity of isolated cultures regularly was checked by light microscopy.

Cultivation of microalgae

Cultures of microalgae were grown under sterile conditions in medium "Chu-13" for 14 days at 25°C, with the supply of carbon dioxide by blowing air containing 1% CO_2 and continuously illuminated by fluorescent white light (200 μmol photons $m^{-2} \cdot s^{-1}$).

TAXONOMY OF MICROALGAE

Taxonomy of isolated algae was identified based on their morphological characteristics, particularly: form of cell, diameter and length of cells, the presence or absence of flagella, spare products and pyrenoids[4,5,11].

Determination of lipids from microalgae

Extraction and determination of intracellular lipids of microalgae were conducted according to the standard protocols in the previous literatures[2,3]. Dry biomass of algae was placed in a glass mortar and sand were added in the ratio 1: 4, i.e. 1 portion of plant biomass and 4 portion glass sand and comminuted until smooth. To the biomass was added methanol containing 10 % DMSO (volume fraction) and stirred 1 hour at room temperature. Then the mixture was centrifuged (3000g, 10 min), and the supernatant was removed. The residua were re-extracted 4 times with a mixture of hexane and diethyl ether (1:1, volume ratio) for 30 min. Each time the mixture was shaken and then centrifuged for 10 min and 3000 g and of the upper organic layer was collected. The organic phases were combined, evaporated to dryness and weighed for microbalance.

RESULTS AND DISCUSSION

Isolation local microalgae

Isolation and production of pure isolates of microalgae is the first step to choose a potential algae for biodiesel production. On the way to a successful isolation of algae from the natural environment, it is necessary to have comprehensive information about their habitat. Therefore, before allocating, the most attention should be paid to the choice of medium for cultivation. At different stages of growth of algae requires different nutrients, so it is necessary to continuously monitor the status of culture. After achievement the required rate of growth of the culture, culture can be grown in conventional nutrient media[14].

Proceeding from the above, to get accumulative cultures of microalgae from different water sources we used minimal medium "Chu-13". To obtain accumulative cultures of microalgae the following conditions are necessary: continuous light illumination, intense bubbling with air containing 2% CO_2 in a sterile condition, culture temperature 28-30°C. During the incubation of samples of water sources in the medium "Chu-13" for 20-25 days visually noticeable association of unicellular green algae appeared.

Water samples from the Syr Darya and Amu Darya of Uzbekistan were received on 30 accumulative cultures of microalgae, which differed in the slurry density and uniformity of the cultures. By dilution method the samples of accumulative cultures were sowed to the 2% agar medium "Chu-13". Within 7 days of incubation on the surface of the agar medium appeared very small, individual colonies of microalgae, and after three

weeks colony size achieved to 2-3 mm. Subsequently, individual colonies of microalgae were grown in a liquid medium for 7-15 days depending on the growth rate of cultures.

Thus, from aqueous samples of Amu darya and Syr darya we isolated and purified 28 local unicellular green algae.

Identification of local microalgae

Chlorococcales algaea fairly large order of green blooms, numbering about 1,200 species [15]. Information on species composition of *Chlorococcales* algae and their distribution in the world is rather limited and incomplete[5].

Taxonomic studies cells showed 15 microalgae refer to the family *Shlorococcaceae*, genus *Chlorococcum* (Figure 1 a, b, c). Cells of algaes are single, sometimes in loose clusters of temporary indefinite shape, ellipsoidal to spherical. The shell is smooth, thickened with age. Goblet cells, spherical, with or without a hole, slightly wavy at the edges wells, usually with one pyrenoid. Spare products starch and oil. Reproduction 2-flagellated zoospores. Size of vegetative cells is 10-22 μ, rarely up to 50 μ in diameter. Genus includes over 100 species, distributed in a variety of aquatic and terrestrial habitats mainly in soils. The most often cited among the aquatic forms are *Chlorococcum humicola* and *Chlorococcum infusionum*[4,5].

Microscopic studies identified 13 local microalgae showed that in their morphological parameters, they belong to the family *Scendesmaceae*, subfamily *Scenidesmoideae*, genus *Scenedesmus* (Figure 1 d, i, f). Cells of algaes flat, straight or slightly curved, one-or two-rowed from 2, 4, 8 cells connected by longitudinal sides parallel to one another, or alternatively, only the ends of the longitudinal sides. Cells are elongate, cylindrical, oval, elliptical to ovate, with rounded, cut or narrowed and blunt ends, often with long spikes or horns at the poles. Length of vegetative cells is 7-15 μ, up to 22 μ. Chloroplast pestered with pyrenoid. In some species in the cytoplasm droplets of oil. Reproduction autospores, one daughter coenobium in each mother cell. The number of species and intraspecific taxa *Scenedesmus* is the most numerous of *Chlorococcales* microalgae[4,5,11].

Lipids of local cultures of microalgae

There has recently been an increased interest in microalgae, which contains a large amount of lipids and fatty acids in membrane structures, and as a reserve energy source[9]. High-performance microalgae, which produces high biomass and lipids is an interesting target

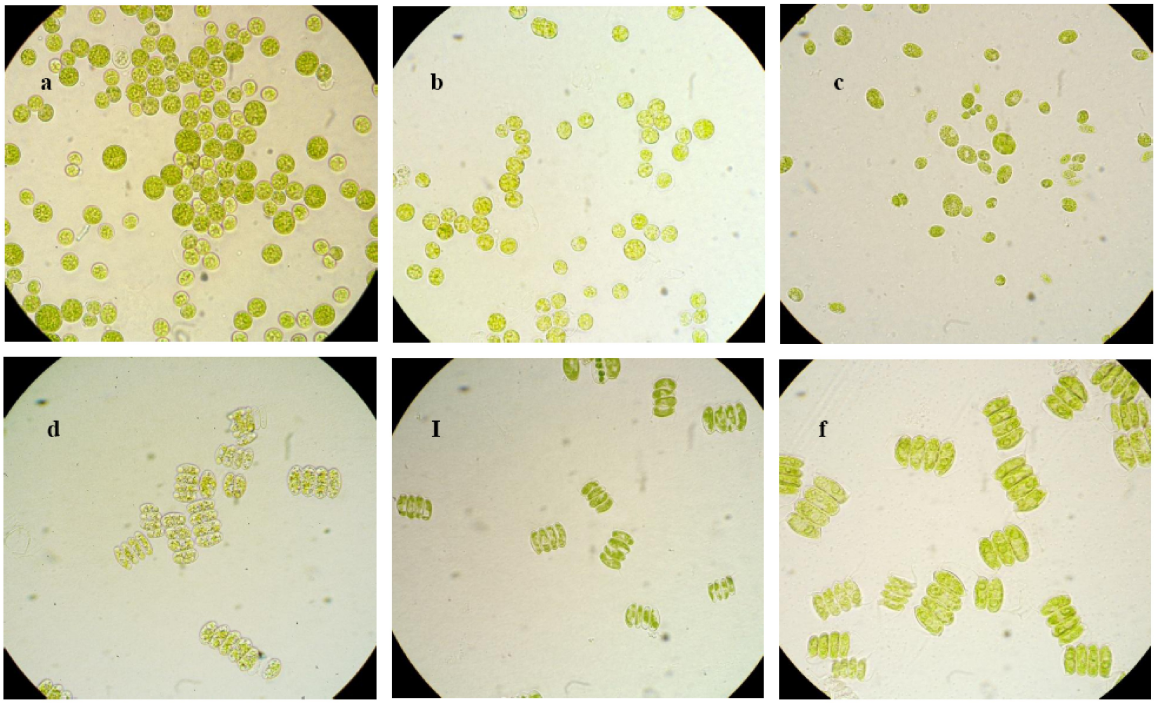

Figure 1 : Light microscopy of microalgae of the genus Chlorococcum and Scenedesmus: a- Chlorococcum sp. 4, b- Chlorococcum sp.8, c- Chlorococcum sp.37, d- Scenedesmus sp.29, i -Scenedesmus sp.37, f - Scenedesmus sp.42

in the search for alternative and renewable biodiesel.

In our research study of the formation of biomass and lipids was conducted in different strains of local microalgae belonging to the genera *Chlorococcum* and *Scenedesmus*.

Cultures of microalgae were grown at sterile conditions on a minimal medium "Chu-13" for 14 days. Biomass Cultures were harvested by centrifugation at 3000g for 30 min, the biomass precipitate is washed 2 times with distilled water. The greatest accumulation of biomass and lipids was found in the cells of cultures *Chlorococcum* sp.4, *Chlorococcum* sp.8, *Chlorococcum* sp.12 and *Chlorococcum* sp.37 (TABLE 1). Air-dry biomass effective local strains of the genus *Chlorococcum* ranged within from 1.29 to 2.29 g/l of medium. The lipid content in these microalgae was 41.8-58.0% of the total dry biomass of strains. Literary data shows that well-known culture

TABLE 1 : Accumulation of biomass and lipid of the strains Chlorococcum grown on medium "Chu-13" for 14 days

Microalgae	Wet biomass, g/l	Dry biomass, g/l	Quantity lipid, %
Chlorococcum sp.4	15.35	2.29	45.8
Chlorococcum sp.5	13.56	1.85	26.5
Chlorococcum sp.7	14.86	2.32	23.0
Chlorococcum sp.8	14.96	2.16	41.8
Chlorococcum sp.12	14.22	1.79	58.0
Chlorococcum sp.16	10.96	2.06	44.8
Chlorococcum sp.18	11.04	1.62	16.8
Chlorococcum sp.19	9.38	1.58	30,5
Chlorococcum sp.20	12.30	1.83	27.5
Chlorococcum sp.29	12.93	1.58	45.6
Chlorococcum sp.32	9.18	1.70	33.9
Chlorococcum sp.37	7.88	1.29	46.8
Chlorococcum sp.38	14.02	2.03	36.4
Chlorococcum sp.39	15.97	2.49	43.7
Chlorococcum sp.40	14.81	2.17	35.2

TABLE 2 : Accumulation of biomass and lipid of the strains Scenedesmus grown on medium "Chu-13" for 14 days

Microalgae	Wet biomass, g/l	Dry biomass, g/l	Quantity lipid, %
Scenedesmus sp. 29	10.92	1.51	48.0
Scenedesmus sp. 31	8.66	1.25	12.5
Scenedesmus sp. 32	6.86	0.85	12.3
Scenedesmus sp. 33	9.66	1.40	12.5
Scenedesmus sp. 34	8.99	0.99	14.6
Scenedesmus sp. 35	11.62	1.55	12.8
Scenedesmus sp. 36	12.51	1.86	14.5
Scenedesmus sp. 37	12.72	2.40	40.3
Scenedesmus sp. 38	11.19	2.79	23.1
Scenedesmus sp. 39	9.23	1.78	24.1
Scenedesmus sp. 40	10.93	2.14	28.7
Scenedesmus sp. 41	10.03	2.04	34.3
Scenedesmus sp. 42	12.14	2.09	30.6

Chlorococcum infusionum grown for 30 days lipid accumulates 28.9% of the total biomass[20].

The strains of microalgae genus *Scenedesmus* formed biomass within the range from 0.866 to 1.277 g/l (TABLE2). A high accumulation of lipids and oils was found in cell cultures Scenedesmus sp.29, *Scenedesmus* sp.37 - 40,3% and 48,0%, respectively. Currently, biodiesel production based on microalgae development encompasses a wide range of industries, as a potential source of renewable energy with economic benefits and environmental advantage. Although there have been isolated and identified many species of microalgae for the production of lipids, there is no consensus about what types provide the highest performance. Different species and types of microalgae function in various geographic and climatic conditions[21].

Thus, aggregate the data, we can conclude that the accumulation of biomass and lipids local microalgae *Chlorococcum* sp.4, *Chlorococcum* sp.12, *Chlorococcum* sp.37, *Scenedesmus* sp.29, *Scenedesmus* sp.37 are highly productive cultures and may serve as a producer of lipid and oils for biodiesel production.

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