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High biomass producers and promising candidates for biodiesel production from microalgae collection IBASU-A (Ukraine)

by

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Abstract

A comparative study was carried out on the growth characteristics of 33 strains of 12 species belonging to genera *Acutodesmus* (7), *Botryococcus* (1), *Chlorella* (5), *Chloroidium* (2), *Desmodesmus* (8), *Euglena* (2), *Monoraphidium* (2), and *Parachlorella* (6) from the Microalgae Culture Collection of the Institute of Botany, NAS of Ukraine (IBASU-A). All high biomass-producing strains considered as promising candidates for biofuel production demonstrated active growth (high maximum cell concentration, specific growth rate and productivity). The most promising strains included *Acutodesmus dimorphus* IBASU-A 251, 252, *Desmodesmus magnus* IBASU-A 401, *D. multivariabilis* var. *turskensis* IBASU-A 398, *Chlorella vulgaris* IBASU-A 189, 192, and *Parachlorella kessleri* IBASU-A 444. Their productivity varied from 0.58 g d.w. l^{-1} to 1.6 g d.w. l^{-1} per day. In general, the cultivation of these strains is considered both as a potential bioresource of feedstock for biodiesel production and other industrial demands.

Key words: biofuel production, IBASU-A collection, strains, biomass producers, specific growth rate, productivity

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Introduction

The search for alternative energy sources relating to the vegetative feedstocks is a major current problem because of the mankind's concern for environmental quality and reduction of the difference in prices of renewable and traditional fuel. In the last two decades, biofuels such as biodiesel as well as bioethanol, biobutanol and biogas have become the real alternatives for fossil energy resources. Initially, the main tendencies of this search were focused on the 1st and the 2nd generations of biofuels produced from industrial crops, namely, oil palm, soybean, sunflower, rape, olive, corn, as well as canola, groundnut, jatropha, etc. (Chisti 2007; Li et al. 2008; Blume et al. 2010). Then, the use of fat-containing food crops such as feedstock for fuel was critically reconsidered and microorganisms, including microalgae, as representatives of the third generation of biofuels, have been recognized as the most promising biological producers (Borowitzka & Moheimani 2007; Chisti 2007; Dismukes et al. 2008; Amin 2009; Gouveia 2011; Barbosa & Wiffels 2013; Borowitzka 2013). In Ukraine, the research based on the use of microalgae biomass as a source for biofuel production has just began in the frame of the scientific project of NAS of Ukraine "Biomass as Raw Material for Fuel" (Korkhovyy et al. 2011; Tsarenko et al. 2011; Zolotaryova & Shnyukova 2010).

The EU countries and the USA, China, Japan, Australia are currently the leaders in the practical use of algae in biofuel processing and production (Gouveia 2011; Barbosa & Wiffels 2013; Borowitzka 2013). Many research and scientific groups have been established with the objective to develop a technical equipment required for the active growing and conversion of algal biomass, as well as the isolation and screening of productive species and strains. The search for high biomass producers characterized by a significant lipid content, a high growth rate and productivity, and the optimization of their culture conditions (sustainable nutrient media, temperature, pH, light intensity and so on) are the priority objectives for effective production of biofuel from microalgae. Unfortunately, the cultured algal species represent only an insignificant part of the world algal flora collection including over 45000 species (more than 5200 in Ukraine). Only about 50 species are known as algae with a high fat content (accumulating lipids in over 10% of their dry weight). They are representatives of Bacillariophyta, Chlorophyta (flagellates and coccoid algae), Haptophyta, Eustigmatophyta, Euglenophyta, Rhodophyta, Streptophyta etc., for example, the members of genera *Botryococccus*, *Chlorella*, *Nannochloris*, *Neochloris*, *Dunaliella*, *Nannochloropsis*, *Monallanthus*, *Isochrysis*, *Tetraselmis*, *Crypthecodinium*, *Cylindrotheca*, *Phaeodactylum*, *Nitzschia* etc. (Chisti 2007; Amin 2009; Zolotaryova & Shnyukova 2010; Richmond & Hu 2013). The presence of oleaginous algal species in different algalculture collections and their active growth under culture conditions are important elements of these research initiatives. The Microalgae Culture Collection of M.G. Kholodny Institute of Botany, NAS of Ukraine (IBASU-A) is one of the largest algal collections in Ukraine. It contains more than 500 strains of 86 species belonging to 40 genera, 9 orders, and 6 classes. The main part of the collection consists of green algae (Chlorophyta), which is the leading taxonomic group of the Ukrainian algal flora. IBASU-A is considered unique as it includes more than 75% of authentic strains isolated from different regions of Ukraine (Borisova & Tsarenko 2004; Microalgae … 2014). At the first stage of our study, the IBASU-A collection was examined to reveal some promising candidates for fuel production. The choice criteria were species ability to accumulate a large amount of lipids, characterized by active growth and tolerance to stress factors and biological contamination. Altogether 33 strains of 12 species belonging to genera *Acutodesmus* (7), *Botryococcus* (1), *Chlorella* (5), *Chloroidium* (2), *Desmodesmus* (8), *Euglena* (2), *Monoraphidium* (2), and *Parachlorella* (6) were screened and their suitable media and growth conditions were determined (Tsarenko et al. 2011; 2012).

The aim of the study is to evaluate the screened strains of microalgal species for biodiesel raw material on the basis of their growth characteristics (cell density, specific growth rate and productivity).

Materials and Methods

The study included 33 strains of IBASU-A belonging to *Acutodesmus dimorphus* (Turp.) P. Tsarenko (strains 251, 252, 254, 344), *A. obliquus* (Turpin) P. Tsarenko (strains 292, 473), *Botryococcus braunii* Kütz. (strain 504), *Chlorella vulgaris* Beij. (strains 189, 190, 192, 326, 452), *Chloroidium saccharophilum* (W. Krüger) Darienko et al. (strains 186, 187), *Desmodesmus armatus* (Chodat) E. Hegew. (strain 270), *D. communis* var. *rectangularis* (G.S. West) E. Hegew. (strain 371), *D. curvatocornis* (Proschk.-Lavr.) E. Hegew. (strain 384), *D. lunatus* (West et G.S. West) E. Hegew. (strain 341), *D. magnus* (Meyen) P. Tsarenko (strains 401, 402), *D. multivariabilis* var. *turskensis* P. Tsarenko et E. Hegew. (strain 398), *D. subspicatus* (Chodat) E. Hegew. et A. Schmidt (strains 310, 407), *Euglena viridis* Ehrenb. (strain 496), *Euglena* sp. (strain 498), *Monoraphidium griffithii* (Berk.) Komark.-Legn. (strain 364), *Monoraphidium* sp. (strain 166), *Parachlorella kessleri* (Fott et Novák.) Krienitz et al. (strains 198, 199, 200, 201, 444), *Scenedesmus obtusus* Meyen (strains 258, 271), *S. raciborskii* Wołosz. (strain 301).

The unialgal or axenic cultures of tested strains were grown under intensive culture conditions. All experiments were conducted in Erlenmeyer flasks of 1000 ml capacity, containing 200 ml of liquid mineral media. The strains were cultivated using the mineral media suitable for each species (Table 1), i.e. the modified Bourrelly (Soeder & Hegewald 1988), Tamiya (Sirenko 1975), 3N BBM (Brown & Bold 1964) and modified Chu 13 (Chu 1942) media. Only the *Euglena* strains were grown in the Hunter medium (Côte et al. 1984) with an addition of yeast extract (0.4 g), thiamine HCl (0.4 mg) and vitamin B_{12} (0.5 μg) instead of liver extract. The pH of each medium was adjusted before autoclaving. According to our previous investigation (Tsarenko et al. 2011), optimal pH was 6.5-8.5 for most of the species under study with the exception of optimal pH 6.0-7.5 for the *Monoraphidium* species, pH 7-8 forthe *Euglena* species, and pH 8.5-9 for *Botryococus braunii.* All the flasks were inoculated uniformly using a suspension of 2-week-old algal cultures and incubated for 7-10 days at temperatures between 26-32°C, constant light with a light intensity of 100 μmol m^{-2} s^{-1} and constant aeration. Initial cell density in the flasks was 5×10^6 cells ml^{-1}. The daily increase in the algal biomass was estimated by the microscopic counting of cell concentration or determination of cell dry weight concentration gravimetrically (Sirenko 1975). Kinetic characteristics of algal growth, such as specific growth rate (μ) and productivity (P), were determined on the basis of previously obtained measurements (Trenkenshu 2005). The productivity of the strains isolated from the Ukrainian territories was compared with the known high biomass producers *Acutodesmus dimorphus* IBASU-A 252 (= *Scenedesmus acutus* CALU 24) and *Chlorella vulgaris* IBASU-A 189 (= CALU 157) obtained fromthe Institute of Microbiology at St-Petersburg University (St-Petersburg, Russia).

Table 1

Comparison of IBASU-A strains selected as promising high biomass producers

Species	Strain (IBASU-A)	Optimal culture conditions			Productivity (g d.w. l^{-1} day^{-1})
		Nutrient medium	pH	Temperature (°C)	
Acutodesmus dimorphus	251–254, 344	Bourrelly	6.5–8.5	26–30	0.6±0.03–1.2±0.17
A. obliquus	292, 473	Bourrelly	6.5–8.5	26–30	0.34±0.02–0.85±0.04
Botryococcus braunii	504	Chu13	8.5–9.0	26–30	1.3±0.19
Chlorella vulgaris	189, 190, 192, 452, 326	Tamiya	6.5–8.5	30–32	0.51±0.09–1.6±0,26
Chloroidium saccharophilum	186, 187	Tamiya	6.5–8.5	30–32	0.48±0.06–0.86±0.04
Desmodesmus armatus	270	Bourrelly	6.5–8.5	26–30	0.39±0.01
D. curvatocornis	384	Bourrelly	6.5–8.5	26–30	0.38±0.01
D. lunatus	341	Bourrelly	6.5–8.5	26–30	0.44±0.14
D. magnus	401, 402	Bourrelly	6.5–8.5	26–30	0.98±0.19–1.2±0.17
D. multivariabilis var. *turskensis*	398	Bourrelly	6.5–8.5	26–30	0.58±0.03
D. subspicatus	310, 407	Bourrelly	6.5–8.5	26–30	0.34±0.04–0.36±0.03
Euglena viridis	486	Hunter	7.0–8.0	30–32	0.30±0.02
Euglena sp.	489	Hunter	7.0–8.0	30–32	0.38±0.03
Monoraphidium griffithii	364	3N BBM	6.0–7.5	26–30	0.29±0.02
Monoraphidium sp.	166	3N BBM	6.0–7.5	26–30	0.31±0.04
Parachlorella kessleri	197–201, 444	Tamiya	6.5–8.5	30–32	0.95±0.12

Results and Discussion

A comparative study on the growth characteristics of 33 screened strains showed that the majority of them is characterized by an active growth, high specific growth rate (μ), and productivity (P). In cultures of the most productive strains of *Chlorella*, *Chloroidium*, and *Parachlorella* species the maximal cell concentrations (B) reached 38–250 × 10^6 cells ml^{-1}. At the same time, the specific growth rate and productivity were 0.55-1.4 day^{-1} and 9.5-72.5 × 10^6 cells ml^{-1} day^{-1}, respectively. In *Acutodesmus* and *Desmodesmus* cultures, the maximal cell concentration reached 26-84.5 × 10^6 cells ml^{-1}, with the specific growth rate and productivity equaling to 0.35-1.2 day^{-1} and 6.4-29 × 10^6 cells ml^{-1} day^{-1}, respectively. Under optimal culture conditions (sustainable nutrient medium, constant light (light intensity 100 μmol m^{-2} s^{-1}), temperature between 30-32°C, aeration, etc.), the growth of algal biomass varied in the range of 0.34 g d.w. l^{-1} to 1.6 g d.w. l^{-1} per day (Table 1). Most of the cultures were characterized by a short term lag phase (1-2 days), i.e. they had a short adaptive period, and the highest rate during active growth. The presence of the bacteria-satellites in the algal cultures of the studied strains did not influence their growth characteristics. The productivity of *Chlorella*, *Chloroidium* and *Parachlorella* strains was the highest and reached 0.51-1.6 g d.w. l^{-1} per day. The productivity of *Acutodesmus* and *Desmodesmus* strains was a little lower and averaged 0.34-1.2 and 0.32-1.2 g d.w. l^{-1} per day, respectively. The least productive algal strains belonged to the genus *Scenedesmus* (*S. obtusus*, *S. rasiborskii*) with the increasing biomass of only 0.11-0.24 g d.w. l^{-1} per day. Other strains belonging to *Monoraphidium* and *Euglena* species had a rather high level of productivity; i.e. up to 0.29-38 g d.w. l^{-1} per day. In general, *Acutodesmus dimorphus* 251, 252, 254 (B – 35-39 × 10^6 cells ml^{-1}, μ – 0.46-0.52 day^{-1}, P – 6.4-9.2 × 10^6 cells $ml^{-1}day^{-1}$), *Desmodesmus magnus* 401, 402 and D. *multivariabilis* var. *turskensis* 398 (B– 84.5 × 10^6 cells ml^{-1}, μ – 0.94-1.2 day^{-1}, P – 22.8-29 × 10^6 cells ml^{-1} day^{-1}), *Chlorella vulgaris* 189, 192 (B – 250 × 10^6 cells ml^{-1}, μ – 1.4 day^{-1}, P – 72.5 × 10^6 cells ml^{-1} day^{-1}), *Parachlorella kessleri* 444 (B – 123.4 × 10^6 cells ml^{-1}, μ – 0.68 day^{-1}, P – 24.3 × 10^6 cells ml^{-1} day^{-1}) were found to be the highest productive strains with the increasing biomass in the range of 0.58 g d.w. l^{-1} to 1.6 g d.w. l^{-1} per day (Table 1, Fig. 1 a-d). These data can be used both for further investigations and for large-scale cultivation and exploitation of the screened strains.

In addition, some data (Fig. 1) showed that an activity of algal growth may be related to taxonomical position of the studied species. For example, the average productivity of species belonging to Trebouxiophyceae (*Chlorella*, *Chloroidium*, *Parachlorella*) was higher than that of Chlorophyceae members (*Acutodesmus*, *Desmodesmus* and *Scenedesmus*). Consequently, the average productivity of *Acutodesmus* and *Desmodesmus* species (Fig. 1b, c) was higher than that of *Scenedesmus* (Fig. 1d). Such information would be helpful in isolating and screening for new promising strains.

According to literature data, some freshwater species of green algae and euglenoids are characterized by the highest content of lipids in their biomass. For example, the representatives of *Scenedesmus* s.l. (including *Acutodesmus* and *Desmodesmus*) are capable of accumulating lipids in their biomass up to 58.3% of dry weight (Mandal & Mallick 2009, Chaundhary et al. 2014), *Choricystis* – 59.3% (Sobczuk & Chisti 2010), *Monoraphidium* – 52% (Griffiths & Dicks 2011), *Chlamydomonas reinhardtii*– 21% and *Euglena gracilis* – 14-20% (Tsarenko et al. 2011). Regardless of the high amounts of lipids, i.e. one of the most important selection criteria, the use of some strains is challenged because of their vulnerability to invasions by other algae, bacteria or fungi. The species of *Chlamydomonas* were excluded from our list of promising strains due to their high sensitivities to contamination by the small-celled green and blue-green algae, which were evident under cultivation.

Moreover, numerous reports in the literature indicate that the *Botryococcus* species contain the highest amount of lipids. These species are considered to be the most promising objects for fuel production because they can accumulate up to 70% of hydrocarbons by dry weight (Chisti 2007; Amin 2009; Zolotaryova & Shnyukova 2010; Barbosa & Wiffels 2013; Borowitzka 2013; Richmond & Hu 2013). They can be found in different climatic zones and have a wide ecological range. In Ukraine, *Botryococcus braunii* and other species of this genus are widespread in different types of water bodies where they actively grow causing algal blooms.

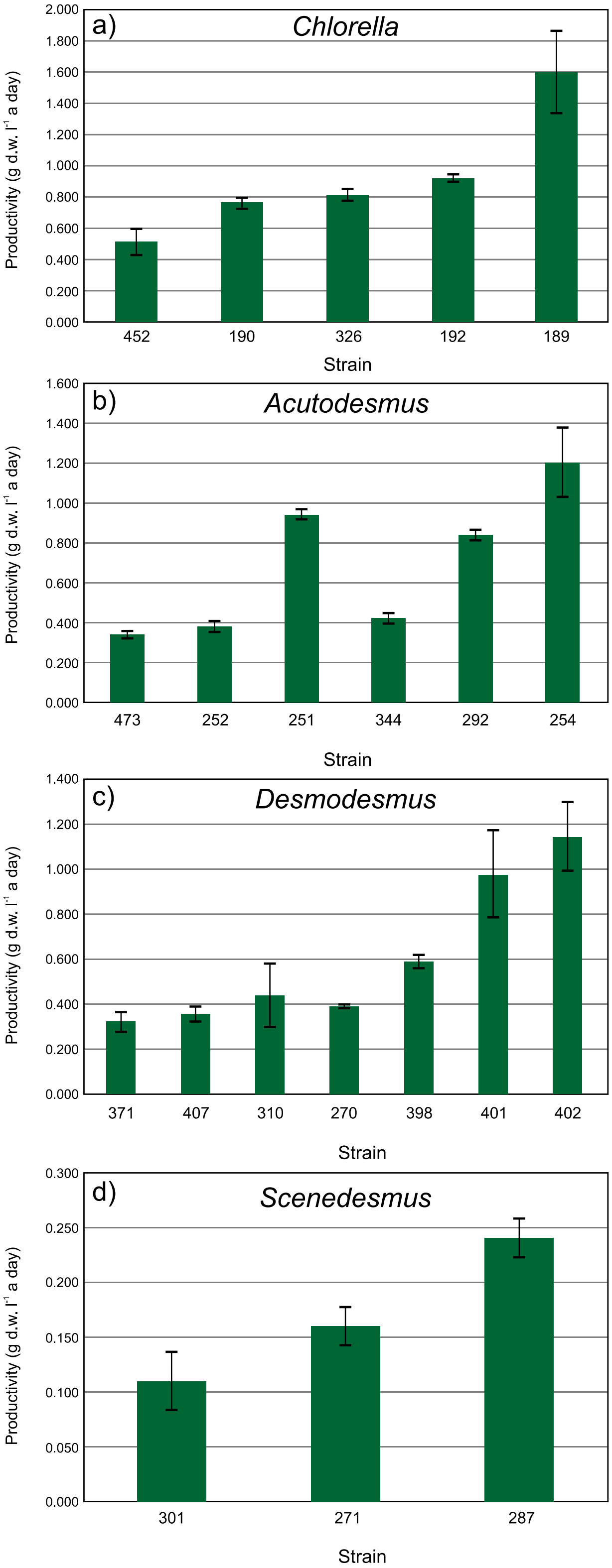

Figure 1

Comparison of productivity of IBASU-A strains: (a) *Chlorella*, (b) *Acutodesmus*, (c) *Desmodesmus*, (d) *Scenedesmus*

Both in nature and under cultural conditions, *B. braunii* demonstrates numerous morphological and chemical varieties. The quantity and quality of its lipids depend on strains, culture conditions, and the growth phase. Our results also showed the high biomass production by strain IBASU-A 504 (1.3 g d.w. l^{-1} per day) and the ability to accumulate lipids up to 46% of dry weight (Tsarenko et al. 2011). However, it has appeared to be highly sensitive to biological contamination and environmental conditions, which caused unstable growth and a considerable decrease in its productivity. These disadvantages may explain why the *Botryococcus* strains have not been widely used on an industrial scale as yet. Nevertheless, the biochemicallyunique ability of these species and prospects of genetic modifications of their genomes may support their use as efficient producers of lipids (Radakovits et al. 2010; Niehaus et al. 2011).

It should also be noted that most of the above-listed species and strains are widely used in important and applied researches and different branches of national economy, food and pharmaceutical industry. For example, *A. dimorphus* IBASU-A252, *A. obliquus* IBASU-A292, *Ch. vulgaris* IBASU-A189-192, *P. kessleri* IBASU-A 197-201 are well-known producers of proteins, vitamins and other bioactive compounds. The application of some strains of *A. dimorphus*, *Ch. vulgaris*, *Chloroidium saccharophilum*, *Euglena gracilis, Monorophidium* (=*Ankistrodesmus*) sp. is highly effective for the development of the closed cycle water use as well as the treatment and post-treatment of different types of waste waters (Lenova, Stupina 1990; Tsoglin et al. 1999).Thereby, the profitability of algae with insufficient commercial value would be raised for account of their multiple-purpose use. However, this requires some additional studies of the specificity of mixed culture cultivation, mixotrophy of green algae, interactions between algae and their accompanying organisms etc.

Conclusions

Our results provided evidence for the presence of 33 high biomass-producing strains in the Microalgae Culture Collection of M.G. Kholodny Institute of Botany, NAS of Ukraine (IBASU-A). Among them, *Acutodesmus dimorphus* IBASU-A 251, 254 (B – 35-

39 × 10^6 cells ml^{-1}, μ – 0.46-0.52 day^{-1}, P – 6.4-9.2 × 10^6 cells ml^{-1} day^{-1}), *Desmodesmus magnus* IBASU-A 401 and *D. multivariabilis* var. *turskensis* IBASU-A 398 (B–84.5 × 10^6 cells ml^{-1}, μ– 0.94-1.2 day^{-1}, P – 22.8-29 × 10^6 cells ml^{-1} day^{-1}), *Chlorella vulgaris* IBASU-A 189, 192 (B – 250 × 10^6 cells ml^{-1}, μ – 1.4 day^{-1}, P – 72.5 × 10^6 cells ml^{-1} day^{-1}), *Parachlorella kessleri* IBASU-A 444 (B – 123.4 × 10^6 cells ml^{-1}, μ – 0.68 day^{-1}, P – 24.3 × 10^6 cells ml^{-1} day^{-1}) are the most promising strains with the increasing biomass in the range of 0.58 g l^{-1} to 1.6 g l^{-1} per day. In general, the cultivation of these strains is considered both as a potential bioresource of feedstock for biodiesel production and other industrial demands. Furthermore, they can be used for the treatment or post-treatment of different types of waste waters and hereby improve the environmental conditions.

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