



# Article **Physiological, Biochemical and Energetic Characteristics of** *Torulaspora globosa*, a Potential Producer of Biofuel

Svetlana V. Kamzolova \* D and Igor G. Morgunov D

G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino Center for Biological Research of the Russian Academy of Sciences, Prospect Nauki 5, Pushchino, 142290 Moscow, Russia; morgunovs@rambler.ru

\* Correspondence: kamzolova@rambler.ru; Tel.: +7-926-414-5620

**Abstract:** It was obtained that the yeast *Torulaspora globosa* VKPM Y-953 is suitable for the production of biodiesel fuel. Zinc plays an important regulatory role in the metabolism of the studied strain. The study of the growth parameters and the fatty acid profile of the yeast *T. globosa* showed that the limitation of its growth by ethanol, at different concentrations of zinc in the medium, considerably influences the chemical composition and the energy content of yeast cells, but not their yield by weight (Yx/s). The increased concentrations of zinc in the medium, in combination with the yeast growth limitation by ethanol, elevated the content of lipids in the cells by 28% and diminished the content of proteins by 14%. At the same time, the limitation of yeast growth by zinc decreased Yx/s and energy ( $\eta_{X/S}$ ) by 2.6 and 3.1 times, respectively. In this case, the content of lipids in cells fell by 72%, while that of proteins rose by 65%. The fatty acid profile of the *T. globosa* cells was used to estimate the main characteristics of biodiesel (iodine value, cetane number, density, and kinematic viscosity). The biomass of *T. globosa* can also be used in agriculture as a feed additive rich in essential amino acids.

Keywords: biodiesel; yeast lipids; Torulaspora globosa; ethanol; zinc

## 1. Introduction

There is no doubt now that lipids are important physiologically active compounds of all living organisms, and not merely reserve materials in cells. Lipids are structural components of crucial cellular organelles, such as membranes, mitochondria and chloroplasts. Lipids affect the active transport of various compounds across outer membranes and the distribution of these compounds inside the cells. They also affect the tolerance of microorganisms to high and low temperatures, acidic conditions, and ionizing radiation; they can also be used as additives to livestock feed and cooking oils, as well as in the manufacture of plant protection and cosmetic products [1]. Lipids participate in the fate of newly synthesized membrane proteins by influencing their translocation, folding, stability, assembly of oligomeric complexes, transport, sorting and enzymatic activity [2]. Microbial lipids can be used for the production of biofuel instead of valuable cooking oils [3–5].

The most promising sources of microbial lipids are yeasts, which can use different carbon sources [6,7]. The range of synthesized yeast lipids can be easily regulated. The advantages of yeasts also include their good growth performance, high lipid yield, and the favorable fatty acid composition of their lipids. All these facts show that yeasts are a promising source of lipids as a crude material for the production of biodiesel on an industrial scale. In comparison with petroleum diesel, biodiesel has some advantages. First, biodiesel is characterized by a relatively high cetane number. Second, it is nontoxic and undergoes almost complete biological decomposition. Third, it can be considered as a renewable fuel, beneficially influencing the  $CO_2$  balance in the atmosphere [3–5].

The most promising producers of lipids are the natural, mutant, and recombinant strains of the yeast genus *Candida*, *Cryptococcus*, *Rhodotorula*, *Trichosporon*, and *Yarrowia* [6–8].



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Lipid synthesis in yeasts occurs under nitrogen limitation and depends on the strain, carbon source, and composition of the growth medium [8–11]. It should be noted that zinc ions play a very important regulatory role in the yeast metabolism. Besides acting as a cofactor for many enzymes, zinc is also required for the structural stability of zinc finger proteins, many of which exert significant control on cellular metabolic processes and stress tolerance [12–15]. Zinc affects many characteristics of yeast, including cell morphology [16], biosynthesis of citric acids [17] and erythritol [18].

As a rule, lipid synthesis in batch culture is a two-phase process: in the first phase, yeast grows until nitrogen is depleted; in the second phase, the excess of carbon source continues to be consumed and accumulates in the form of storage lipids [1,6–8]. However, it was shown that the yeast *Cryptococcus terricolus* produces lipids in parallel with the synthesis of other components [19]. A unique property of intensive lipid synthesis during the phase of active growth was also found in the yeast *Torulaspora globosa* (syn. *Debaryomyces globosus*) [20–22]. However, studies of *Torulaspora globosa* as a lipid producer are still insufficient.

The aim of this work was to study the physiological, biochemical and energetic characteristics of the yeast *Torulaspora globosa* VKPM Y-953 and define the role of zinc in the metabolism of the studied strain.

## 2. Materials and Methods

Experiments were carried out with the yeast Torulaspora globosa VKPM Y-953.

The yeast was cultivated in a chemostat regime using an ANKUM-2M fermentor (working volume 7 L). The cultivation medium contained (mg/L):  $(NH_4)_2SO_4$ , 870; MgSO<sub>4</sub>·7H<sub>2</sub>O, 300; CaCl<sub>2</sub>·6H<sub>2</sub>O, 30; KH<sub>2</sub>PO<sub>4</sub>, 1200; yeast extract Difco, 500; FeSO<sub>4</sub>·7H<sub>2</sub>O, 9; MnSO<sub>4</sub>·5H<sub>2</sub>O, 1.3; CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.6; and CoCl<sub>2</sub>·6H<sub>2</sub>O, 1.2. The growth substrate was rectified ethanol at a concentration of 4% volume. The concentration of zinc ions was varied from 0.005 to 2.8 mg/L. Initially, batch mode was carried out on a mineral medium of the above composition. When the culture reached a stationary phase (cessation of titration), the chemostat mode was switched on with a flow rate of 0.05 h<sup>-1</sup>. When the culture reached a steady state under given experimental conditions, cultivation was continued until the effluent volume reached three times that of the culture volume.

The cultivation temperature was maintained at an optimal level for this yeast strain (28.0  $\pm$  0.1 °C). The pH of the medium during fermentation was maintained at a level of 5.2 by automatic addition of the necessary volume of 85 ml/L NH<sub>4</sub>OH solution. The aeration rate was 1.3–1.5 L/L min and the agitation rate was 400 rpm.

The cell biomass was determined by passing an amount of the culture liquid through a paper filter. The filter cake containing yeast was dried at 105  $^{\circ}$ C to a constant weight.

The content of carbon, hydrogen and nitrogen in the biomass was determined using a C,H,N-1 Gas Analyzer (KOVO, Czech Republic). To estimate ash, the biomass was burned in a muffle furnace. The content of oxygen was calculated as the difference between dry biomass and the total content of C, H, N and ash.

Lipids were evaluated as described in the inventor's certificate [23]. The methyl esters of fatty acids were analyzed using a Chrom-5 gas–liquid chromatograph equipped with a flame ionization detector and a column (2 m  $\times$  3 mm) packed with a stationary phase representing 15% Reoplex 400 on Chromaton N-AW (0.16–0.200 mm). The column temperature was 200 °C.

Biomass samples were prepared for chromatography as follows: the samples were treated with acetyl chloride and methanol and then boiled to evaporate the solvents. The residue was treated with hexane. The content of lipids in the biomass were calculated from the sum of fatty acids using docosane ( $C_{22}H_{46}$ ) and heptadecanoic acid as the internal standards.

The major characteristics of biodiesel were estimated using the following empirical formulas [24].

Iodine value (*IV*) (expressed in mg  $I_2/100$  g) was calculated as:

 $IV = \sum (254 \cdot Ni \cdot wi)/Mi$ , where Ni is the number of double bonds in a fatty acid with index "*i*", Mi is its molecular mass, and wi is the content of this acid in the sum of all fatty acids.

Cetane number (*CN*) was calculated by the formula:

 $CN = 46.3 + 5458 / \sum SV - (0.255 \cdot IV)$ , where SV is the saponification value (expressed in mg KOH/g-oil), estimated as SV = 560 (% wi) / Mi.

Density ( $\rho$ ) was evaluated in kg/m<sup>3</sup> as follows:

 $\rho = \sum \rho_i \cdot wi$ , where  $\rho_i = 0.8463 + 4.9/Mi + 0.0118 \cdot Ni$ .

Highest heating value (HHV) was calculated in MJ/kg by the formula:

 $HHV = 49.43 - 0.041 \cdot SV - 0.015 \cdot IV.$ 

Kinematic viscosity was estimated in mm<sup>2</sup>/s as described in the work [25].

Data presented in the tables are the mean values of three experiments and two measurements for each experiment; standard deviations were calculated (SD < 10%).

### 3. Results and Discussion

3.1. The Relation of Growth Efficiency and Lipid Formation in T. globosa

Table 1 summarizes the results of the measurement of growth efficiency and the analysis of the chemical composition of yeast cells grown under limitation by ethanol at different concentrations of zinc ions in the cultivation medium.

Table 1. Indices of growth and composition of *T. globosa* VKPM Y-953 biomass at different Zn concentrations.

Indices	Zn Concentration (mg/L)					
	0.005	0.1	0.28	2.8		
Biomass yield by mass $(Y_{X/S})$ (%)	$20.9 \pm 1.05$	$56.7\pm0.23$	$56.7\pm0.17$	$55.5\pm0.23$		
Energy capacity of biomass $(Q_B)$ (kJ/g)	$16.6\pm0.26$	$19.49\pm0.3$	$22.31\pm0.3$	$22.55\pm0.22$		
Biomass yield by energy $(\eta_{X/S})$ (%)	$13.8\pm0.12$	$37.6\pm0.12$	$43.1\pm0.53$	$42.6\pm0.29$		
Lipids (% of biomass)	$9.2\pm0.12$	$20.0\pm0.6$	$26.4\pm0.6$	$27.8\pm0.53$		
Protein (% of biomass)	$37.96 \pm 0.12$	$26.82\pm0.28$	$20.23\pm0.26$	$23.03\pm0.34$		
C (% of biomass)	$42.52\pm0.36$	$46.62\pm0.21$	$50.81\pm0.5$	$51.04 \pm 0.29$		
H (% of biomass)	$6.94\pm0.36$	$7.38\pm0.21$	$7.95\pm0.3$	$8.01\pm0.2$		
N (% of biomass)	$7.6\pm0.23$	$5.1\pm0.2$	$4.06\pm0.1$	$4.52\pm0.36$		
O (% of biomass)	$41.22\pm0.29$	$36.36\pm0.23$	$34.07\pm0.3$	$32.65\pm0.22$		
Lipid yield by mass $(Y_{L/S})$ (%)	4.8	11.2	14.1	17.2		
Lipid yield by energy $(\eta_{L/S})$ (%)	6.5	15.2	19.1	23.3		

As seen in Table 1, the nonlimiting concentrations of zinc (2.8, 0.28 and 0.1 mg/L) almost did not affect the yield of *T. globosa* VKPM Y-953 cells grown under ethanol limitation (Yx/s from 55.5 to 56.7%). At the same time, the limiting concentration of Zn (0.005 mg/L) reduced Yx/s to 20.9% (i.e., by 2.6 times).

One can suggest that the yeast cell modifies its metabolism in response to the change in the growth-limiting factor. The metabolisms of yeast cells grown with similar growth rates but limited by different growth factors (for example, nitrogen and zinc) may be very different. The physiological response of cells to different limitations is still poorly understood and hence difficult to predict.

When the concentration of zinc in the medium was reduced from 2.8 to 0.1 mg/L and then to the limiting concentration of 0.005 mg/L, the energy content of the biomass ( $Q_B$ ) decreased from 22.55 to 19.49 and 16.6 kJ/g, respectively (Table 1).

The energy yield of the biomass ( $\eta_{X/S}$ ) also depended on the concentration of zinc in the cultivation medium. Thus, when the concentration of zinc was reduced from 2.8 to 0.1 and then to 0.005 mg/L,  $\eta_{X/S}$  decreased from 42.6 to 37.6 and 13.8%, respectively (i.e., by 3.1 times). At the same time, the dependence between Yx/s and  $\eta_{X/S}$  was not detected. Indeed, when the concentration of zinc in the medium was reduced from 0.28 to 0.1 mg/L, the cell yield (Yx/s) did not change and remained at 56.7%, while  $\eta_{X/S}$  decreased by 16%. In other words, the energy yield  $\eta_{X/S}$  characterizes the efficiency of substrate conversion

into cell biomass better than Yx/s does, because the first parameter takes into account the energetic value of not only the growth substrate, but also the biomass.

When the concentration of zinc was increased from 0.1 to 2.8 mg/L, the content of lipids in the biomass rose from 20.0 to 27.8% (by 28%), while the content of protein decreased from 26.82 to 23.03% (by 14%). At the growth-limiting concentration of zinc (0.005 mg/L), the content of lipids in the *T. globosa* cells fell to 9.2% (by 72%), and that of proteins increased to 37.96% (by 65%).

There are few data in the literature on the ability of *T. globosa* to synthesize lipids in great amounts. The closely related yeast *Debaryomyces etchellsii* BM1 can synthesize lipids in amounts comprising up to 25.9% of the biomass when assimilating various inexpensive carbon sources, such as deproteinized whey, waste waters from olive oil mills and confectionery factories, and expired beverages [26]. A higher lipid accumulation (45.7% of biomass) was observed for the strain *T. globosa* YV5/2 cultivated in media with a surplus of glucose [27], the maximum lipid accumulation (up to 90% of biomass) being observed for the genetically modified strain of *Y. lipolytica* [28].

As seen in Table 1, there is a correlation between the contents of lipids, proteins and particular chemical elements in the biomass of *T. globosa* VKPM Y-953. As the protein content increases in the biomass from 23.03% to 37.96%, so does the nitrogen content from 4.52% to 7.6%. As the content of carbon and hydrogen decrease from 51.04% to 42.52% and 8.01% to 6.94%, respectively, the content of lipids drops from 27.8% to 9.2%. This result is explained by the fact that lipids are reduced compounds basically containing carbon and hydrogen atoms. The content of carbon and hydrogen in the biomass correlates with its energy content ( $Q_B$ ). Hence, QB is mainly determined by the lipid content of the biomass. This conclusion is consistent with the fact that lipids are the most energetic compounds in the biomass. For reference, the energy content of lipids, proteins, nucleic acids and glycogen comprises 9.92 kJ/g, 5.07 kJ/g, 2.45 kJ/g, and 4.05 kJ/g, respectively [29].

To evaluate the efficiency of lipid formation, we estimated the lipid yield by mass  $(Y_{L/S})$  and by energy  $(\eta_{L/S})$ , the latter parameter characterizing the portion of the chemical energy of the growth substrate passed to the synthesized lipids. As seen in Table 1, when the concentration of zinc decreased from 2.8 mg/L to 0.1 mg/L and then to the limiting concentration of 0.005 mg/L,  $Y_{L/S}$  fell from 17.2% to 11.2% and 4.8%, respectively. The maximum yield of lipids by energy ( $\eta_{L/S} = 23.3\%$ ) was observed for the *T. globosa* VKPM Y-953 cells grown in the medium with 2.8 mg/L Zn, which decreased to 15.2% and 6.5%, respectively, in the yeast cells grown at 0.1 and 0.005 mg/L Zn.

Thus, it follows from Table 1 that the increased concentrations of zinc in the medium promote lipid synthesis in *T. globosa* VKPM Y-953 by 28%, while its growth-limiting concentration inhibits lipid synthesis by 72%. This conclusion contradicts the literature data on other yeasts. For example, the growth limitation of *Lipomyces starkeyi* by zinc promoted lipid synthesis [30]. Some authors believe that the growth-limiting concentrations of zinc promote lipid synthesis in the yeasts (such as *Trichosporon pullulans*) potentially capable of intense lipid synthesis, while exerting no effect on the yeasts (such as *Candida valida*) with potentially weak lipid synthesis [31].

## 3.2. Changes in the Fatty Acid Profile of Lipids

The suitability of yeasts for lipid production greatly depends on the fatty acid profile of their lipids [24,32–34].

As seen in Table 2, irrespective of the cultivation conditions used, lipids in *T. globosa* VKPM Y-953 cells mainly contain unsaturated fatty acids with a predominance of palmitoleic ( $C_{16:1}$ ) (29.2–41.7%) and oleic ( $C_{18:1}$ ) (30.0–42.2%) acids. These results agree with the literature data on the lipids of the closely related yeast *D. etchellsii* [26].

Fatty Acids (% from Sum of Fatty Acids)	Zn Concentration (mg/L)					
	0.005	0.1	0.28	2.8		
Myristic acid (C <sub>14:0</sub> )	$0.8\pm0.02$	$2.1\pm0.2$	$3.3\pm0.47$	$2.50\pm0.21$		
Myristoleic acid ( $C_{14:1}$ )	$0.7\pm0.02$	$0.6\pm0.08$	$1.0\pm0.09$	$0.40\pm0.08$		
Palmitic acid ( $C_{16:0}$ )	$16.5\pm1.0$	$18.6\pm1.2$	$23.8\pm0.3$	$24.60\pm0.23$		
Palmitoleic acid ( $C_{16:1}$ )	$29.2\pm1.2$	$41.7\pm2.35$	$40.7\pm0.54$	$37.5\pm2.63$		
Stearic acid ( $C_{18:0}$ )	$3.3\pm1.2$	$2.1\pm0.8$	$1.2\pm0.08$	$2.5\pm0.2$		
Oleic acid ( $C_{18:1}$ )	$42.2\pm2.2$	$34.9\pm4.0$	$30.0\pm1.1$	$32.5\pm0.7$		
Linoleic acid ( $C_{18:2}$ )	$7.3\pm0.9$	trace	trace	trace		
Sum of unsaturated fatty acids	79.4	77.2	71.7	70.40		

 Table 2. The effect of zinc on fatty acid composition.

Cultivation conditions had little effect on the qualitative fatty acid composition of lipids, but influenced it quantitatively (Table 2). In particular, the reduction in zinc concentration in the medium from 2.8 mg/L to 0.1 mg/L increased the degree of lipid unsaturation at the expense of a higher content of palmitoleic acid ( $C_{16:1}$ ). The growth of *T. globosa* VKPM Y-953 at the limiting concentration of zinc (0.005 mg/L) caused the appearance of the essential linoleic acid (7.3% of the sum of fatty acids). The important biological role of unsaturated fatty acids has recently been reviewed by some authors [32–34]. Being cofactors of some enzymes, they possess high physiological activity, and are also involved in oxidative phosphorylation, the regulation of activity of various enzymatic systems and the permeability of cellular and mitochondrial membranes.

## 3.3. Calculated Characteristics of Biodiesel on the Basis of T. globosa Lipids

The profile of fatty acids, including the length and the degree of unsaturation of their carbon chains, is very important for the calculation of the physicochemical characteristics of biodiesel [3,24,32–36].

To characterize the biodiesel that can be produced on the basis of *T. globosa* lipids, we calculated its potential parameters, such as iodine value, cetane number, density and highest heating value. These parameters must meet the requirements of the respective international standards, namely, ASTM D6751 (USA), EN 14,214 (EU), CAN/CGSB-3.524 (Canada), and IS 15607-05 (India) [24].

Table 3 summarizes the results of such calculation in comparison with the standards of biodiesel defined by ASTM D6751 and EN 14214.

	T. globosa VKPM Y-953			Limits of Biodiesel Standards		
Indices	Zn (mg/L)			ASTM D6751	FN 14214	
	0.005	0.1	0.28	2.8		
Iodine value ( $IV$ ) (mg I <sub>2</sub> /100 g)	81.1	73.9	68.9	67.3	-	≤120
Saponification value (SV) (mg KOH/g-oil)	208.7	212.6	214.2	212.9	-	-
Cetane no.(CN)	51.81	51.33	51.14	51.29	$\geq 47$	$\geq 51$
Density ( $\rho$ ) (kg/m <sup>3</sup> )	0.87	0.87	0.87	0.87	-	0.86-0.90
Kinematic viscosity (mm <sup>2</sup> /s)	4.99	5.0	5.0	5.0	1.9-6.0	3.5-5.0
High heating value $(HHV)$ (MJ/kg)	39.32	39.26	39.22	39.27	-	-

Table 3. Biodiesel properties of T. globosa VKPM Y-953 lipids.

Iodine value (IV) is the main parameter of fuels containing unsaturated fatty acids, which characterizes their chemical stability. In contrast to saturated fatty acids, unsaturated ones have a low chemical stability [3,35,36]. As seen in Table 3, the iodine value (IV) for *T. globosa* VKPM Y-953 varies from 67.3 to 82.1 mg I<sub>2</sub>/100 g, and thus does not exceed the threshold value restricted from above (120 mg I<sub>2</sub>/100 g).

Similar iodine values (74.13–80.95 mg  $I_2/100$  g) were obtained for the yeasts Y. *lipolytica*, L. *starkeyi*, and *Rhodosporidium toruloides*, whereas the iodine values of the microalgae *Chlorella vulgaris* and *Chlorella sorokiniana* grown in media with glucose (160.90 and 133.3 mg  $I_2/100$  g, respectively) exceeded the threshold value of 120 mg  $I_2/100$  g [36].

Cetane number (CN) is an indicator of the combustion speed of diesel fuel and the degree of compression needed for ignition. It plays a similar role for diesel fuels as an octane number does for gasoline. Generally, diesel engines operate well with a CN from 45 to 55 [36]. Fuels with a lower cetane number have longer ignition delays, requiring more time for the fuel combustion process to be completed [35]. Hence, higher-speed diesel engines operate more effectively when run on fuel with a higher cetane number. As seen in Table 3, the cetane number of biodiesel that can be produced from *T. globosa* VKPM Y-953 lipids is 51.14–51.84, and this meets the requirements of the European and American standards EN 14214 and ASTM D6751, with minimum cetane numbers of 51 and 47, respectively.

Density is also an important characteristic of fuel that correlates with other relevant parameters (viscosity, cetane number, heating value) [3,24,35,36]. For example, fuel with a higher density usually has a higher heating value. Generally, biodiesel is denser than petroleum diesel [24]. Diesel density is restricted by a range of 0.86–0.90 g/cm<sup>3</sup> in the European standard EN 14214, and has no restrictions in the American standard ASTM D6751. As seen in Table 3, the biodiesel that can be produced from *T. globosa* VKPM Y-953 lipids meets the requirements of the European standard EN 14214.

The next parameter, kinematic viscosity, influences the efficiency of fuel atomization and the size of droplets. Low-viscosity fuels reduce pressure in the fuel injection system and cause pumps and injectors to leak; this results in reduced power and efficiency in diesel engines. High-viscosity fuel increases flow resistance in pipes, manifolds and injectors and decreases the efficiency of fuel pumps and injectors. The combustion of fuel decreases, causing the appearance of harmful products from incomplete combustion, increased fuel consumption and engine wear [24,35]. Usually, the kinematic viscosity of biodiesels is higher than that of petroleum diesels by 10–15%, due to their higher molecular mass and specific structure. The ranges of the kinematic viscosity of diesel fuels defined by ASTM D6751 and EN 14214 are 1.9–6.0 and 3.5–5.0 mm<sup>2</sup>/s, respectively. As seen in Table 3, the kinematic viscosity of biodiesel that can be produced from *T. globosa* VKPM Y-953 lipids (4.99–5.0 mm<sup>2</sup>/s) meets the requirements of both standards, although it lies on the upper limit of the European standard EN 14214.

Highest heating value (HHV) characterizes the fuel efficiency of engines [3,24,35,36]. This parameter is defined by neither of the standards ASTM D6751 and EN 14214. The calculated value of HHV for the *T. globosa* VKPM Y-953 lipids is about 39 MJ/kg and corresponds to HHV values of biodiesel from the oleaginous yeasts *Y. lipolytica*, *L. starkeyi* and *R. toruloides* [36].

Thus, according to calculations based on the fatty acid profile of *T. globosa* VKPM Y-953, the biodiesel that can be produced from the biomass of this yeast meets the requirements of the relevant standards.

#### 3.4. Effect of Zinc on the Amino Acid Profile

*T. globosa* VKPM Y-953 can also serve as the source of proteins as well as lipids. The data shown in Table 4 indicate that the amino acid profiles of yeast cells grown under different cultivation conditions are almost identical. The highest contents were observed for aspartic acid (2.020–3.560% of dry biomass), alanine (1.430–2.540% of dry biomass), glutamic acid (5.960–7.410% of dry biomass), arginine (1.220–2.750% of dry biomass), and serine (1.740% of dry biomass).

As seen in Table 4, the decrease in the concentration of zinc from 2.8 mg/L to 0.1 mg/L diminishes the content of isoleucine (by 12.9%), leucine (by 9.0%), phenylalanine (by 6.4%), tryptophan (by 24.5%), valine (by 5.9%), and histidine (by 8.8%), but enhances the content of arginine (by 27.2%). The growth-limiting concentration of zinc (0.005 mg/L) favors the synthesis of isoleucine, leucine, lysine, phenylalanine, tyrosine, cystine and methionine.

The maximum proportion of essential amino acids (41.52% of all amino acids) was observed when the growth of *T. globosa* VKPM Y-953 was limited by zinc.

Aming Agid (% from Sum of Agids)	Zn Concentration (mg/L)				
Amino Acid (% from Sum of Acids) –	0.005	0.1	0.28	2.8	
Glutamic acid	19.52	27.07	27.68	25.88	
Asparagine	9.378	9.172	9.540	8.771	
Lysine	7.271	6.376	5.981	6.644	
Leucine	8.430	7.084	7.118	7.729	
Valine	5.611	4.959	5.339	5.254	
Threonine	5.005	4.884	5.141	4.950	
Serine	4.505	4.474	4.498	4.559	
Tyrosine	3.030	2.983	3.065	2.909	
Alanine	6.691	5.593	6.179	6.209	
Isoleucine	4.426	3.691	3.856	4.168	
Phenylalanine	4.031	3.878	3.856	4.125	
Glycine	4.294	3.393	3.658	4.038	
Histidine	3.003	2.834	3.015	3.083	
Methionine	1.475	1.156	0.989	1.129	
Cysteine	1.212	1.119	1.384	1.563	
Sum of aromatic AAs	7.060	6.861	6.920	7.034	
Sum of sulphur AAs	2.687	2.274	2.373	2.692	
Trypthophan	1.027	1.081	1.384	1.346	
Arginine	7.244	7.271	6.179	5.297	
Proline	3.846	2.983	1.137	2.345	
Sum of essential AAs	41.517	37.211	38.112	39.818	

Table 4. Amino acid content of T. globosa biomass.

#### 4. Conclusions

The novelty of this study lies in the study of the physiological and biochemical characteristics of *T. globosa* VKPM Y-953, with a unique ability to synthesize lipids in parallel with the growth of the culture. It was found that the concentration of zinc ions causes significant changes in the composition of the biomass. An increased zinc content led to an increase in lipids and a decrease in protein content, which correlated with the content of macronutrients in the biomass. It has been demonstrated that biodiesel, produced from the lipids of *T. globosa* VKPM Y-953, meets the existing European and American standards. The biomass of the studied strain can also be used as a feed additive in agriculture.

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