Establishment of Growth Conditions for Cultivation of The Microalga *Trachydiscus minutus* at Laboratory Scale

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ABSTRACT

The alga *Trachydiscus minutus*, while being a relatively new subject of laboratory research, is not yet involved in a real biotechnological development. The aim of this study was an elaboration of the intensive growth conditions. A new medium specifically designed for the cultivation of the alga was composed, and the reasons were discussed. The use of the new medium leads to at least 10% higher growth than media previously used. The maximal growth rate of the alga remains the same, but the growth is comparatively higher before achieving plateau-phase, which is due to a better composition. The new medium is significantly cheaper per 1 kg algal biomass. Attention was paid to precise light and temperature conditions. The separation of *Trachydiscus* biomass is discussed as an important part of its biotechnology. The biochemical composition of the alga at these conditions is described. The paper is targeted to micro-enterprises in accordance with their limited resources.

Key words: algae, cultivation, nutrition medium, *Trachydiscus minutus*

Introduction

An upsurge has been seen in the area of research and development of microalgae, and intensive seeking and study of new species suitable for photoautotrophic biotechnology is going on all over the world. The alga *Trachydiscus minutus*, being a relatively new subject of laboratory research, is not yet involved in a real biotechnological development. Nevertheless, harking back to the preliminary experience, one could regard *Trachydiscus minutus* as a prospective alga worth growing on a large scale [1]. For example, its ability to produce dense laboratory cultures, its abundant lipid content and high percentage of arachidonic and eicosapentaenoic acids are useful qualities [2, 3, 4, 5]. Promising evidence has even been reported that the content of eicosapentaenoic acid could be enhanced adding spermine [6].

There is not a nutrition medium specially designed for cultivation of *T. minutus*. At present, at least three different media are in use, which had initially been meant for other purposes. The universal Zehnder's nutrition medium [7] applied in concentrations of $\times 0.5$, $\times 1$, $\times 3$ has been used [2, 3, 4, 8]. The medium of Zachleder and Šetlík [9] has been used in other studies [3, 10]. The medium of Bischoff and Bold [11] was preferred in one case [12]. The first of these media was composed for cultivation of cyanobacteria, the second and third mostly for green algae. Though suitable for these purposes, these media have shown some drawbacks while cultivating xanthophycean *Trachydiscus minutus*, concerning both proportion and kind of salts. In order to reveal the real photosynthetic ability of *T. minutus* alga, and thereby its biotechnological potential, finding an appropriate nutrition medium fit to the peculiarities of the alga, was one of the aims of this paper.

The yield depends on the temperature, and dealing with algae, the estimation of the optimum temperature is one of the first tasks [2, 3, 4, 13, 14]. The optimum temperature estimation is related to the exact quantification of dry algal biomass. There is some difference between the temperatures regarded as optimal (Table 1). The same conclusions could be made about the effects of light intensity and they are worth discussing.

It is well known that powerful and expensive centrifuges are one of the substantial hindrances on the way to large-scale microalgal production. Usually, algae-biomass-pursuit concerns small and even micro-enterprises which have limited resources. It is worth mentioning that many algae, such as green *Chlorella*, *Scenedesmus*; red *Porphyridium* and *Rhodella*, or yellow-green *Nannochloropsis* though producing valuable biomass, are hard to separate from the medium. In laboratory conditions they are separated for 20 min at 6000-8000 revolutions per minute (rpm), about 4000 g [15, 16]. Industrial production of microalgae requires powerful centrifuging of about 7000 rpm and up to 12000 g [17]. A tentative evaluation of the gravimetric separation of *T. minutus* was another objective of this work.

Materials and methods

Cultivation of algae

The alga *Trachydiscus minutus* (strain Lukavsky and Přibyl 2005/1) was grown in laboratory as a mono-algal culture in 200 cm³ glass flasks (5 × 1 cm cross-cut), a glass vessel 8 dm³ in a vertical layer (50 × 27 × 6 cm) and a covered pond of 2 m² and 7 cm depth. The optimum temperature, 26 °C, was estimated in the range 14 - 40 °C using a temperature gradient unit. Uninterrupted illumination 300 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ from luminescent lamps (5 × 40 W), and continuous

bubbling with $3 \text{ cm}^3 \cdot \text{s}^{-1}$ air enriched with 0.5% CO_2 was used. The covered pond was illuminated with solar light.

The initial algal density was $0.6 \text{ g} \cdot \text{dm}^{-3}$. Test cultures were cultivated using a newly composed nutrition medium with NaNO_3 as it is given in Table 2, and urea $1 \text{ g} \cdot \text{dm}^{-3}$. Control cultures were cultivated using the proportion of Zehnder's, (Z) nutrition medium with 3 times higher concentration [2, 7]. The medium described by Zachleder and Šetlík [9] was used as a parallel control. The state of monoculture was checked every day microscopically. Besides, the fatty acid composition was used as a taxonomic marker [18, 19]. Algae were inoculated in a sterile box, in sterilized cultivation flasks and media, and filters $0.45 \mu\text{m}$ were used on the way of the bubbling gas.

Analytical procedures

Algal biomass was centrifuged 20 min at 3000 rpm, washed and dried at 105°C to constant weight. Growth was measured gravimetrically every 24 h. The analysis of the pigment content was measured spectrophotometrically, according to the McKinney method [20]. Total protein was analyzed according to Lowry's method [21], and carbohydrates according to Dubois [22].

Fresh biomass was extracted with chloroform/methanol 2:1, 3 times for 0.5 h under reflux. The extract was evaporated *in vacuo* and the residue was re-extracted with chloroform to obtain the total lipids, which were estimated gravimetrically. The total lipids were converted to fatty acid methyl esters, (FAME) by heating in methanol containing 6% m/m anhydrous HCl at 60°C for 1.5 h. The FAME were extracted with hexane and purified by TLC on silica gel with hexane - diethyl ether (10:1 v/v). Gas chromatography of FAME was carried out on a column 2.5% SE52 / 10% DEGS at 195°C on a Perkin-Elmer gas chromatograph with flame ionization detector.

Triacylglycerols (TG) were separated from the lipids by thin layer chromatography (TLC) on silica gel G with hexane - diethyl ether (3:1 v/v). The spots were identified using sunflower oil as a reference substance and quantified densitometrically. Plates were sprayed with H_2SO_4 /ethanol (2:3 v/v) and heated at 110°C to visualise the spots.

The estimation of the necessary centrifugal force to separate the biomass from the medium was conducted gravimetrically by measuring the residual algal or bacterial density.

Results

A nutrition medium for cultivation of *Trachydiscus minutus* was composed of new salt proportions compared to the proportions of the previously used media (Tables 2, 3). The medium was prepared in 2 variants that differ only in the nitrogen source – urea or sodium nitrate. Comparisons between the variants showed that highest growth was achieved in the medium with NaNO_3 (Tables 2, 3). This new medium ensured at least 10% higher growth rate (Fig. 1, Table 4) than the control.

Our experiments showed that good growth was achieved only if the initial algal density was higher

than $0.5 \text{ g} \cdot \text{dm}^{-3}$ dry matter, regardless of the media used. Furthermore the lag-phase lasted undesirably longer at lower algal density. The highest growth rate we observed was with NaNO_3 at about day 6 and 7 and corresponded to algal density of approximately $4 \text{ g} \cdot \text{dm}^{-3}$. The growth decreased and practically stopped at day 12 due to complete assimilation of the nutrients available. A mean daily growth of $0.6 \text{ g} \cdot \text{dm}^{-3}$ was achieved with the newly composed medium with sodium nitrate, while the growth of the control was $0.55 \text{ g} \cdot \text{dm}^{-3}$, and the growth of the variant with urea was $0.45 \text{ g} \cdot \text{dm}^{-3}$ dry matter, as average values for the whole period.

The growth of the alga in urea medium, though slower, is acceptable for practical application. The lag-phase was slightly longer and the growth stopped on day 14, which was 2 days longer as compared to the cultivation in NaNO_3 medium. A satisfactory outcome was achieved in a variant of the medium with NH_4NO_3 where the algal growth was similar to growth in urea medium (data not shown).

We used the medium of Zachleder and Šetlík [9] as a parallel control, as well. It was found that this medium allowed maximum production of $7.0 \pm 0.2 \text{ g} \cdot \text{dm}^{-3}$ dry algal biomass which is similar to the maximum biomass achieved in the Zehnder's medium [7].

The optimum temperature was estimated to be $26 - 27^\circ\text{C}$. The growth decreases significantly under temperatures below 25°C and above 30°C . The growth rate remains constant at light intensity above $300 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. *Trachydiscus minutus* requires a lower light intensity, compared to other commercially grown algae. The intensity of solar light over $1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ influences negatively the yield and the biochemical composition of the alga expressed in a decrease percentage of eicosapentaenoic acid.

The stage of separation of the algal biomass from the liquid is shown on Table 6. The full separation of *T. minutus* cells was achieved at 1000 g. After centrifuging the biomass produced in control medium, a clearly distinguishable bacterial layer was found, which was not found, or presented too scarcely while working with the new medium. In the control medium 3Z, the weight of total dry bacteria was $0.36 \pm 0.04 \text{ g} \cdot \text{dm}^{-3}$ at algal density $8 \text{ g} \cdot \text{dm}^{-3}$ dry weight.

The whole experiment showed an advantage of *T. minutus*, namely that the cells did not stick to the flasks and cultivation vessels. The algal cells show a tendency to aggregate and fall to the bottom of the flask or the pond during growth at density over $2 \text{ g} \cdot \text{dm}^{-3}$.

The biochemical composition of *Trachydiscus minutus*, obtained under the optimal conditions is given in Table 7.

Discussion

The maximal algal growth rate, it means in the inflexion point, is obviously one and the same in the new medium and the medium 3Z used as a control (Fig. 1). The same is it in the Zachleder and Šetlík medium used as a second control. Nevertheless, using the new medium, the growth at the plateau phase continues up to 10% higher yield. The explanation

could be one: there is a betterment of the new medium salt proportion. As for the growth of *Trachydiscus*, it remains twice slower compared to the usual growth of the green algae *Chlorella*, *Scenedesmus* or *Coelastrum* [23, 24, 25], and it could hardly be more quick. The alga *Trachydiscus* is nearly twice rich in fats and lipogenesis in general is a highly energy-consuming process. Making relatively rich triacylglycerol reserve is a natural property of *Trachydiscus*.

According to the ash composition assay, the proportions of micro- and macroelements of most cyanobacteria and microalgae are similar, and it is a preliminary tentative base when a new media is composed [26, 27, 28].

As a rule, algae that prefer alkaline nitrate as nitrogen source grow more slowly compared to those grown on ammonium ions or urea [29]. The reduction of the nitrate requires energy and time. Choosing the kind and concentration of the nitrogen source is a top priority. Acceptable nitrogen sources are limited, and they include NH_4NO_3 , urea, nitrates of alkaline or alkaline earth metals. Although algae grow successfully on NaNO_2 [30], this source is unsuitable because of its oxidative properties and hazardous side effects. The fitting nitrogen source depends on the activity of either enzyme nitrate reductase or urease.

Media, composed with KNO_3 [9], where Na^+ is totally excluded, have a small advantage, namely because the exhausted solution remaining after separating the biomass is appropriate for agricultural watering of higher plants. The salts KH_2PO_4 or K_2HPO_4 as sources of PO_4^{3-} maintain a concentration of K^+ which fully meets algal requirements. At high concentration levels of KNO_3 , the active ion transport supplies K^+ to the algal cells with an unusually high, almost stressful concentration. Taking in consideration the higher price of KNO_3 and its lower nitrogen content it is clear that this salt is not the proper one for large-scale biomass production.

The normal total protein content of *Trachydiscus* varies between 30 - 45% of its dry mass [2, 4]. The concentration of 4.042 g.dm^{-3} KNO_3 , described in the medium of Zachleder and Šetlík [9] (see Table 2), corresponds to 7 g.dm^{-3} dry algal mass with a normal protein content and therefore, both stoichiometric reckoning and our experiments confirmed that a higher algal density at 4.042 g.dm^{-3} KNO_3 was a groundless expectation.

The cultivation with urea is an acceptable approach, worthy of being further evaluated. Urea, unlike alkali nitrates, is a source about three times more abundant in nitrogen and is fully metabolized. After biomass separation, the solution can be used to water plants without restriction, i.e. it implies both environment-friendly behaviour and good husbandry.

The nitrogen/phosphorus ratio of algae is not very different as compared to other plants, so that the concentration of the source of PO_4^{3-} ought to be conformed to this of the N-source. The new nutrition medium is richer in PO_4^{3-} than the medium of Bischoff & Bold [11] and the Zehnder's medium [7], as shown

in Table 2; the medium of Zachleder & Šetlík contains unnecessary high excess of PO_4^{3-} .

MgSO_4 is another salt, whose concentration has often been either underestimated or overestimated. It provides both Mg^{2+} and SO_4^{2-} , and a small excess of SO_4^{2-} remains at the end of cultivation. Applying MgSO_4 at a concentration higher than biological requirements is justified when it is a buffer: in cases of higher concentration of alkali nitrate and non-intensive growth. The medium of Tamiya [31] is one example. It is a wrong practice to add MgSO_4 in excess when algae are grown intensively. The aftermath of higher MgSO_4 concentration is precipitation of $\text{Mg}_3(\text{PO}_4)_2$ which is centrifuged together with algal biomass. Obviously, this leads to both disbalance of the medium and aggravation of biomass quality. On the other side, the Zehnder's medium [7] and the medium of Bischoff & Bold [11] have an insufficient amount of MgSO_4 . Magnesium, being component of the chlorophyll molecule, can be reckoned after spectrophotometrically pigment quantification. The content of chlorophyll "a" increases significantly at the end stage of the cultivation at a sufficiency of magnesium. The chlorophyll "a" content was 1.3% (per dry weight) in cultures grown under the 3Z medium, being 2.5% as a mean value with the new medium when use sodium nitrate, and up to 3.5% when use urea as nitrogen source. The difference can even be observed visually: cultures grown under Zehnder's medium have a yellowish hue, while cultures grown with the new composition look healthy and green.

Even if NaHCO_3 is not added in the medium, it is subsequently formed as an aftermath of algal growth, and its concentration increases proportionally to the biomass. As a matter of fact, the consumption of NaNO_3 leads to appearance of NaHCO_3 . The experiment has shown that some initial concentration of NaHCO_3 has a positive effect on algae. The question concerning NaHCO_3 is usually bound to the activity of the enzyme carbonic anhydrase. This enzyme has a real significance at very low concentration of CO_2 [32, 33]. In the case of intensive cultivation, CO_2 is supplied in some excess, giving a full expression of the photosynthetic abilities of the alga. Availability of NaHCO_3 facilitates the yield of biomass in case of uneven CO_2 supply due to the low solubility of the gas and its quick consumption. An equimolar quantity of NaHCO_3 is useful to be added as a buffer when NH_4NO_3 is nitrogen source [23].

Chloride ions are almost always available in the natural water and little heed has been paid to their concentration in the media. Nevertheless, a deficiency of Cl^- is perceptible at laboratory cultivation when pure salts and distilled water are used. The growth rate decreases significantly when cultivation is carried out without Cl^- , and it is especially visible at higher algal density (unpublished results). Here ought to be mentioned that the ratio of ions $\text{Cl}^- / \text{Na}^+$ is about 11:1 (w/w), or 7:1 as equivalents in dry algal or cyanobacterial biomass [28, 34, 35]. There is a stoichiometric deficiency of ions Cl^- in the medium of Zachleder and Šetlík [9] they thoroughly absent in the

Table 1. Temperature and light intensity applied at *T. minutus* cultivation.

Refference	Temperature, °C	Light intensity, $\mu\text{mol.m}^{-2}.\text{s}^{-1}$
Sun light, covered pond		
[8]	26	> 1000
Laboratory cultivation		
[2;8]	26	175
[3]	28	450
[13]	27	160
[4]	25	2×132
[10]	28	500
[14]	28	470

Table 2. Nutrition media macroelements mg.dm^{-3}

Macroelements	Zehnder [7]	Bischoff & Bold [11]	Zachleder & Šetlík [9]	New medium
NaNO_3	467	250	-	2000
KNO_3	-	-	4042	-
KH_2PO_4	-	175	340	-
K_2HPO_4	31	75	-	250
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	25	75	988	266
Na_2CO_3	21	-	-	-
NaHCO_3	-	-	-	100
NaCl	-	25	-	50
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	59	-	-	40
$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	-	25 / $2\text{H}_2\text{O}$	10.96	-
$\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$	-	-	-	20
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	-	50	-	-
Fe-EDTA	10	-	-	-
Fe/Na-EDTA	-	-	18	-
$\text{Na}_2\text{-EDTA}$	-	49.8	-	-

Table 3. Nutrition media microelements mg.dm^{-3}

Microelements	Zehnder [7]	Bischoff & Bold [11]	Zachleder & Šetlík [9]	New medium
H_3BO_3	6.18	0.114	3.09	2
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	2.87	0.882	1.43	2
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2.23	-	1.18	2
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	-	0.144	-	-
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	2.49	0.157	1.24	0.4
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	1.96	-	0.88	0.04
MoO_3	-	0.071	-	-
$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	2.81	-	1.4	0.004
$\text{CoNO}_3 \cdot 6\text{H}_2\text{O}$	-	0.049	-	-

Table 4. Daily yield of *T. minutus* in the new media with nitrogen source, g.dm⁻³

Day	Control, 3 × Zehnder's medium [7]		New medium with NaNO ₃		New medium with urea	
	Dry weight ^a	Daily yield	Dry weight ^b	Daily yield	Dry weight ^c	Daily yield
0	0.6 ± 0.07	-	0.6 ± 0.05	-	0.61 ± 0.03	-
1	0.8 ± 0.2	0.2	0.8 ± 0.1	0.2	0.50 ± 0.06	-0.11
2	1.2 ± 0.2	0.4	1.2 ± 0.1	0.4	0.45 ± 0.01	-0.5
3	1.7 ± 0.3	0.5	1.9 ± 0.1	0.7	0.51 ± 0.01	0.06
4	2.4 ± 0.3	0.7	2.6 ± 0.1	0.7	0.90 ± 0.08	0.39
5	2.9 ± 0.2	0.5	2.8 ± 0.3	0.2	1.63 ± 0.05	0.33
6	3.8 ± 0.1	0.9	3.8 ± 0.1	1.0	2.33 ± 0.07	0.70
7	4.7 ± 0.0	0.9	4.8 ± 0.1	1.0	3.18 ± 0.04	0.85
8	5.1 ± 0.1	0.4	5.2 ± 0.1	0.4	4.08 ± 0.09	0.90
9	5.6 ± 0.1	0.5	5.7 ± 0.1	0.5	4.98 ± 0.09	0.90
10	6.1 ± 0.1	0.5	6.3 ± 0.1	0.6	5.78 ± 0.08	0.80
11	6.9 ± 0.1	0.8	6.9 ± 0.1	0.6	6.55 ± 0.05	0.77
12	7.2 ± 0.1	0.3	7.8 ± 0.0	0.9	6.76 ± 0.09	0.21

Mean value and standard deviation of: a - 6 experiments, b - 6 exp., c - 3 exp.

Table 5. Fatty acid composition (% m/m) of *T. minutus*

Fatty acid	%
12:0	0.8 ± 0.3
14:0	23 ± 5
16:0	10 ± 4
16:1	10 ± 4
16:2	2 ± 1
18:0	0.5 ± 0.2
18:1	6 ± 3
18:2	10 ± 5
18:3 ^{6,9,12}	0.5 ± 0.3
18:3 ^{9,12,15}	5 ± 2
20:4	6 ± 2
20:5	40 ± 5

Table 6. Centrifugation of algal culture of *T. minutus*

RPM, min ⁻¹	G-force × g	Time, min	Result
1000	156	5	Sedimentation of about 95% of algae
1000	156	10	Sedimentation of about 99% of algae
2000	625	0	Total sedimentation of algae and negligible sedimentation of bacteria
3000	1408	0	Sedimentation of up to 50% of bacteria
4000	2505	10	Sedimentation of 95% of bacteria
5000	3915	10	Total sedimentation of all cells,

Table 7. Composition of dry biomass of *T.**Svetoslav et al.,*

Substances	% (m/m)
Proteins	42 ± 3
Carbohydrates	25 ± 5
Lipids	26 ± 4
Triacylglycerols	5 ± 1
Esterified fatty acids	10 ± 1
Eicosapentaenoic acid	3 ± 1
Chlorophyll a	2 ± 1
Carotenoids	0.95 ± 0.05

Figure 1. Growth of *T. minutus*.

Z medium, and are sufficient with the new proportion. Additionally, the concentration of Cl^- is several fold higher compared to Na^+ in a plenty of well-studied higher plants [37]. This is a token of the significance of Cl^- for the photosynthesizing organisms at all and worth studying separately.

The concentration of the salts of microelements (Table 3) in the Šetlík [36] medium is a value expressed as an integer number-fold by the molecule mass of the salt. The medium Zachleder & Šetlík [9] is 0.5 concentration of Šetlík [36]. Obviously, this approach does not express the natural nutritional requirement of an organism. That is why the microelements were composed in a new proportion taking into consideration the mineral composition of a living cell [37] and previous experience [38] with algae.

There is a suggestion concerning all nutrition media in which *T. minutus* has been cultivated, namely that the Fe-source has to be used in EDTA-chelated form. Our experience with cyanobacteria, red and green algae, and *T. minutus*, has shown that chelating is useless. The non-chelated Fe-salt could be oxidized and even precipitated. Nevertheless, at a constantly high CO_2 concentration, Fe-salt will be re-dissolved, thus maintaining sufficient concentration of Fe-ions. Algae cultivation does not require chelating neither Fe-ions nor ions of other microelement, and this research inertia was influenced by medical and pharmaceutical studies, in accordance with requirements of human and animal organisms, receiving microelements in a proper form with their food. There are many cases, for example the practically insoluble FeS_2 , which can lead to appearance of Fe-ions in measurable concentration at CO_2 -bubbling [39].

A medium for intensive cultivation of microorganisms, in particular microalgae, in addition to proper nutrition qualities, is better to have a low physical density which facilitate centrifuging the biomass. The new medium answers to this quality with its physical density of 1.0027 g.dm^{-3} compared to the density 1.0054 g.dm^{-3} of medium Zachleder and Šetlík [9]. The new medium, producing about 10 % more biomass, having composed with smaller amount of salts and cheaper NaNO_3 instead of KNO_3 , is obviously preferable from economic point of view.

As for the temperature, it is satisfying to be between 25 - 28 °C, which is relatively easily maintained even when growing in higher volumes and areas. Finding the optimum temperature is bound to accurate measuring of the growth, which is preferable to be done gravimetrically. Spectrophotometrically derived data can be tentative: the ratio pigments / dry biomass is not always linear due to the variations of the composition; the algae make aggregates; the background is not a constant value. These facts lead to errors, which increase proportional to the algal density.

The daily light intensity is higher than the requirement of *Trachydiscus*. As a matter of fact, the alga is under light stress especially about noon hours. Darkening the photosynthesizing area in order to decrease the light intensity is not recommendable. The progressive attitude of mind seeks to increase the ratio area/volume

by folding the volume in order to illuminate the main part of the photobioreactor with about $500 - 600 \mu\text{mol.m}^{-2}.\text{s}^{-1}$.

In the nature *Trachydiscus* has not been found as a dense culture, being scattered amid ubiquitous *Chlorella* or *Scenedesmus* is not easily recognizable. As we have said, in laboratory, the state of the dense cultures of *Trachydiscus* as mono-algal ones, save microscopically, was controlled according to their qualitative fatty acid composition (Table 5). Being genetically determined, the fatty acid composition (carbon atoms and double bonds number) is rather acceptable chemotaxonomic marker [18, 19]. Here we have to share the observation that *Trachydiscus* is easily yielding to contamination with other algae, that is why all precautions are obligatory. This is one reason more to cultivate the alga in closed photobioreactor, but not in open pond. The concomitant bacteria are non-pathogenic, aerobic, and occupying the ecological niche, suppressing the development of pathogenic bacteria [40].

On the ground of gravimetric analyze, and bearing in mind the theory of resemblance of mechanical and particularly hydrodynamic processes, the following conclusions could be made, based on data shown in Table 6. The centrifuge radius, earth acceleration and the algal mass are constant values. The centrifugal force is variable and directly proportional to n^2 , where n is revolutions per time. The centrifugal force at 3000 rpm is 9 times higher, as compared to 1000 rpm. A 33-seconds stay of the algal biomass in the centrifuge at 3000 rpm which corresponds to 5 min at 1000 rpm is enough for an acceptable separation. A continuous flow centrifuge is a simple implementation and suffices a good performance being significantly cheaper. Gravimetric separation is also aided by the combination of both higher algal density and lower physical density of the liquid, achieved with the new medium.

Generally, our research efforts regarding *Trachydiscus minutus* confirmed its useful biotechnological qualities, and valuable biomass (Table 7). The alga is worth being in the visual field of the potential manufacturers of biomass. In the context of contemporary phycological pursuits, we would like to make it plain that the driving force of our work was not microalgal biodiesel, but algae as food and feed additives and pharmaceuticals, as we have documented elsewhere [41]. The valuable biomass is worth eating and not burning. The high content of polyunsaturated fatty acids is alimentary advantage and diesel-fuel-disadvantage. Nevertheless, concerning the biofuel question, we welcome a taking part of this alga in the integrated heterotrophic-photoautotrophic processes for methane production. Based on other useful qualities of *Trachydiscus*, the work will continue with a main point of purification of methane and CO_2 mineralization.

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