



## NOTES

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### CARBOHYDRATES AS AN ORGANIC SUBSTRATE FOR MICROALGAE *TISOCHRYSIS LUTEA* (HAPTOPHYTA) UNDER CONDITIONS OF LABORATORY CULTURE

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The possibility was studied of using carbohydrates in order to optimize the cultivation process of microalgae *Tisochrysis lutea* (Haptophyta). The effect of D-galactose, glucose, and sucrose at concentrations of 100 and 200 mg·L<sup>-1</sup> on the dynamics of *T. lutea* abundance was analyzed. As found, adding of all the studied carbohydrates stimulated microalgae growth, with sucrose at a concentration of 200 mg·L<sup>-1</sup> having the most pronounced effect.

**Keywords:** *Tisochrysis lutea*, cell abundance, carbohydrates, aquaculture

Microalgae are a food source for various groups of hydrobionts. Among marine microalgae, *Tisochrysis lutea* is of great interest as a food object for invertebrates: mussels, oysters, scallops, and sea cucumbers. Moreover, it is used to feed larvae of salmon, flounder, and representatives of other fish families. Controlling the growth parameters of algae cultures (enriching media composition, introducing nutrient substrates, etc.) results in growth acceleration (Bigagli et al., 2018). Currently, one of the objectives of cultivation is to obtain high abundance of microalgae in a short time.

The aim of this work is to assess the effect of carbohydrates on the growth of marine microalgae *Tisochrysis lutea*.

## MATERIAL AND METHODS

The study object was the culture of unicellular algae *T. lutea* (Haptophyta) – MBRU\_Tiso-08 strain from the Marine Biobank resource collection at NSCMB FEB RAS (Efimova et al., 2016). *T. lutea* was cultivated on the nutrient medium *f*, with D-galactose, glucose, and sucrose added at concentrations of 100 and 200 mg·L<sup>-1</sup>, at a temperature of +20 °C, a light intensity of 50 μmol·m<sup>-2</sup>·s<sup>-1</sup>, in a visible light area, and a light/dark cycle 12 h : 12 h. Microalgae were grown in 250-mL Erlenmeyer flasks with 100 mL of nutrient medium, under conditions of a periodic cultivation mode. As a control, an alga grown on the medium *f* with no carbohydrates added was used. The experiments lasted 21 days. *T. lutea* abundance was counted under a microscope in a Goryaev chamber. Algae biomass (wet weight)

was calculated by the formula from (Levich et al., 1997). The experiments were carried out in three biological replicates. Mean values and standard deviations were calculated. The data was statistically processed in Microsoft Office Excel 2007.

## RESULTS AND DISCUSSION

Up to the 18<sup>th</sup> day, D-galactose adding at both concentrations stimulated *T. lutea* growth and biomass increase. On the 21<sup>st</sup> day, these values were lower than the control ones at 100 mg·L<sup>-1</sup> of D-galactose (Table 1).

**Table 1.** Effect of carbohydrates on *Tisochrysis lutea* abundance and biomass

Day	Control	D-galactose		Glucose		Sucrose	
		100 mg·L <sup>-1</sup>	200 mg·L <sup>-1</sup>	100 mg·L <sup>-1</sup>	200 mg·L <sup>-1</sup>	100 mg·L <sup>-1</sup>	200 mg·L <sup>-1</sup>
Cell abundance, × 10 <sup>4</sup> per mL							
0	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2
2	24.5 ± 0.1	29 ± 0.3	23.8 ± 0.1	21.2 ± 0.2	21.7 ± 0.4	23.7 ± 0.4	32.3 ± 0.9
4	26.5 ± 0.3	34.5 ± 0.5	29.8 ± 0.4	31.7 ± 0.5	25.5 ± 0.5	35.0 ± 0.7	31.2 ± 1.2
7	33.8 ± 0.3	52.8 ± 1.0	51.0 ± 1.5	36.7 ± 0.7	59.2 ± 0.8	49.4 ± 0.5	47.0 ± 0.9
9	64.5 ± 0.6	84.9 ± 0.8	93.2 ± 0.9	74.2 ± 0.9	74.7 ± 1.0	70.4 ± 0.7	69.9 ± 1.2
11	102.8 ± 0.7	121.0 ± 1.2	121.0 ± 1.3	96.8 ± 0.3	97.5 ± 0.9	246.6 ± 1.0	294.6 ± 1.0
14	122.0 ± 0.4	134.6 ± 1.6	149.8 ± 1.4	142.0 ± 1.3	168.5 ± 1.2	157.2 ± 0.9	165.9 ± 0.9
16	167.0 ± 0.9	176.2 ± 0.9	178.1 ± 0.9	191.2 ± 0.8	194.5 ± 0.8	219.7 ± 1.5	219.0 ± 0.7
18	479.8 ± 1.2	634.3 ± 1.3	585.4 ± 1.7	424.8 ± 1.4	434.2 ± 1.3	745.3 ± 1.8	792.8 ± 1.6
21	587.2 ± 1.4	509.0 ± 1.7	581.7 ± 1.0	607.8 ± 1.0	574.5 ± 1.5	750.3 ± 0.9	833.6 ± 0.5
Biomass, mg·L <sup>-1</sup>							
0	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
2	0.7 ± 0.2	0.9 ± 0.4	0.7 ± 0.2	0.6 ± 0.5	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.4
4	0.8 ± 0.4	1.0 ± 0.6	0.9 ± 0.5	1.0 ± 0.6	0.8 ± 0.4	1.0 ± 0.4	1.0 ± 0.6
7	1.0 ± 0.9	1.6 ± 0.4	1.6 ± 0.7	1.1 ± 0.5	1.8 ± 0.8	1.5 ± 0.5	1.4 ± 0.8
9	1.9 ± 0.3	1.5 ± 1.2	1.6 ± 0.9	2.2 ± 0.8	2.2 ± 0.4	2.1 ± 1.2	2.1 ± 0.8
11	3.1 ± 1.1	3.7 ± 0.7	3.7 ± 1.1	2.9 ± 1.1	3.0 ± 1.2	7.5 ± 1.1	8.9 ± 1.3
14	3.7 ± 1.1	4.0 ± 1.2	4.4 ± 1.5	4.3 ± 0.9	5.1 ± 1.0	4.8 ± 0.8	5.0 ± 1.5
16	5.0 ± 0.9	5.4 ± 0.9	5.4 ± 0.9	5.8 ± 1.3	5.9 ± 0.7	6.7 ± 0.9	6.7 ± 1.2
18	14.5 ± 1.8	19.2 ± 1.3	17.8 ± 1.9	12.9 ± 1.7	13.1 ± 1.6	22.7 ± 1.9	24.1 ± 2.0
21	17.8 ± 1.6	15.5 ± 1.8	17.7 ± 1.3	18.5 ± 1.8	17.4 ± 1.3	22.8 ± 2.2	25.3 ± 1.9

**Note:** mean values and standard deviations are given.

With glucose adding in both concentrations, an increase in *T. lutea* abundance and biomass was registered on the 7<sup>th</sup>–16<sup>th</sup> days of the experiment (see Table 1). Sucrose stimulated algae growth, especially from the 11<sup>th</sup> day (see Table 1). The most pronounced effect was recorded at 200 mg·L<sup>-1</sup>. As shown in the literature, microalgae growth intensifies with organic substrates adding (*inter alia* carbohydrates): in *Chlamydomonas reinhardtii* and *Haematococcus pluvialis* (Jeon et al., 2006), in *Chlorella sorokiniana* and *Chlorella* sp. (Heredia-Arroyo et al., 2010), and in *Nannochloropsis* sp. (Hu & Gao, 2003).

**Conclusion.** D-galactose, glucose, and especially sucrose adding results in an increase in *T. lutea* abundance and biomass. The effect of carbohydrates on microalgae growth is dose-dependent. Hence, carbohydrates adding is a promising method of *T. lutea* cultivation.

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## УГЛЕВОДЫ КАК ОРГАНИЧЕСКИЙ СУБСТРАТ ДЛЯ МИКРОВОДОРОСЛИ *TISOCHRYSIS LUTEA* (НАПТОРФУТА) В УСЛОВИЯХ ЛАБОРАТОРНОЙ КУЛЬТУРЫ

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Исследована возможность применения углеводов для оптимизации процесса культивирования микроводоросли *Tisochrysis lutea* (Haptophyta). Проанализировано влияние D-галактозы, глюкозы и сахарозы в концентрациях 100 и 200 мг·л<sup>-1</sup> на динамику численности популяции *T. lutea*. Установлено, что добавление всех изученных углеводов стимулировало рост микроводоросли, однако наибольший эффект оказала сахароза в концентрации 200 мг·л<sup>-1</sup>.

**Ключевые слова:** *Tisochrysis lutea*, численность клеток, углеводы, аквакультура