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Spirulina: growth in continuous and batch bioreactors and response to stress conditions

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Abstract. *Spirulina platensis* is a cyanobacterium with high biotechnological potential. Optimization of the cultivation conditions of spirulina takes place constantly, to increase the yield of this valuable crop. An important economic indicator of the process is an increase in the yield of biomass and a decrease in the cost of its production. For example, avoiding the need to cultivate spirulina at elevated temperatures with at least equal biomass yield. The purpose of this work was to develop and compare methods for cultivating *Spirulina platensis* in two bioreactors, continuous and batch ones, and to assess the resistance of spirulina cells to environmental stress caused by the presence of household chemicals. It was shown that, at room temperature, the wet biomass yield was higher in the bioreactor than with batch cultivation in flasks. However, a decrease in temperature leads to a decrease in the number of colony forming units. The presence of compounds such as phosphates and phosphonates from household chemicals in the cultivation environment negatively affects the survival of *Spirulina* cells, which reflects the general trend of a decrease in the number of microorganisms as a result of environmental pollution with surfactants.

1. Introduction

Different species of cyanobacteria (formerly blue-green algae) inhabit almost all environments — from soil to fresh and sea waters, as well as such extreme habitats as hot springs, soda and salt lakes, etc. [1]. The production of microalgae is growing at an intensive rate throughout the world, among them the microalgae *Chlorella* spp. and the cyanobacterium *Spirulina* spp. are most widely used. *Spirulina* is used as feed for animals and poultry, in the food and pharmaceutical industries to obtain algaline flour (ecobroad), polyunsaturated fatty acids (omega-3, omega-6) physiologically necessary for humans, biologically active substances (astaxanthin, phycocyanin), new generation antibiotics. According the Hunger Project, in November 2017, out of 7.6 billion of the population in the world, 815 million are in a state of chronic hunger. Among them, almost three quarters of the population is directly dependent on agriculture and similar activities [2]. At the same time, it is noted that an increase in the productivity of agricultural products inevitably entails an increased load on natural resources and the environment [3].

Plant-based proteins are currently the main source of protein for food and feed. Expansion of sown area, change in sowing frequency and increase yields can help meet the growing demand for food; but,



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yields can approach the ceiling in terms of optimization [4]. All of the above testifies to the extremely promising use of algae as an alternative food for humans and animal feed.

For thousands of years, humans have been using microalgae as a component of their diet. Most experts in the field of nutrition, agricultural production, ecology believe that the use of microalgae can provide humanity with a complete supplement to the traditional diet and reduce the stress load on terrestrial agricultural resources [5]. The use of microalgae has a number of advantages. So, for example, the protein yield from microalgae is 4-15 tons / ha / year compared to 1.1 tons / ha / year for wheat, 1-2 tons / ha / year for legumes and 0.6-1.2 tons / ha / year for soybeans [6].

Crop production accounts for approximately 75% of all freshwater in the world [7]. Moreover, animal protein sources consume 100 times more water compared to plant sources for equivalent protein extraction. In addition, marine microalgae can be grown without fresh water and arable land, further maximizing the resources needed to produce additional terrestrial food crops [6].

The population of many developed countries totally suffers from diseases of civilization - hypertension, diabetes, metabolic syndrome, etc. caused by high-calorie, but unbalanced diet, which lacks vitamins, PUFAs, antioxidants, etc. At the same time, a number of microalgae species contain high-grade proteins, carbohydrates, lipids. Microalgae are also a source of vitamins A, B1, B2, B6, B12, C and E, trace elements and minerals such as iron, magnesium, calcium and iodine [8,9]. The study of the microbiota of the human digestive tract has led to the understanding that it plays a key role in the body's resistance to a number of diseases, including infectious ones. Over the past two decades, there have been many reports that the stability and consistency of the composition of the intestinal microbiota is largely determined by the presence in food of low molecular weight polysaccharides - the so-called dietary fiber. Seaweed and microalgae are very promised as sources of dietary fiber [10].

Therefore, every year there is an increasing interest in the topic of using microalgae as a valuable food additive. So, for example, chlorella is positioned as an effective treatment and prevention of diseases such as Alzheimer's disease and cancer. Therefore, the global chlorella market is growing rapidly and stood at US \$ 138 million in 2016 and is expected to reach US\$ 164 million by 2021 [11]. In addition, due to their rapid photoautotrophic growth and high speed of biomass accumulation, these cyanobacteria are now considered as important renewable energy alternatives for petroleum-based fuels [12]. These organisms are used to produce various biotechnological products with high added value [13-15]. In its natural habitats, *Spirulina* is an important part of the ecosystem in wastewater, playing the role of absorbing heavy metal ions, preventing them from entering the human body and having a beneficial effect on the ecology of the reservoir. The absorbing properties of dry biomass of *Spirulina* and live strains of cyanobacteria or blue-green algae *Spirulina platensis* and *Aphanothece flocculosa* were studied extensively. For example, *Spirulina platensis* was used to adsorb cadmium and nickel ions [16]. Al-Homaidan et al. [17] used dry *S. platensis* biomass for the biosorption of copper ions from aqueous solutions. A soluble in water *S. platensis* extract was used to remove hexavalent chromium ions from industrial wastewater [18]. Four types of biomass with different biochemical composition of *Arthrospira (Spirulina) platensis* were used to remove copper and nickel ions [19]. All studies recommended using spirulina biomass as a biosorbent for heavy metal removal.

The aim of this work was to study the effect of household chemicals on the survival of *Spirulina* and the development of a phytobioreactor for continuous cultivation of *Spirulina* without reducing the amount of biomass.

2. Materials and methods

2.1. Organisms and cultivation conditions.

For cultivation, an algologically pure culture of *Spirulina platensis* obtained from "Smart Spirulina" was used. Two photobioreactors, I and II, were built for *Spirulina* cultivation.

The photobioreactor I was consisted of two modules - a spiral photosynthetic module and a module for carbon dioxide bubbling, biomass harvesting and growth medium renewal. The spiral module with a volume of 30 l was made in the form of a spiral from a transparent silicone tube with an inner diameter

of 20 mm. The inner diameter of the spiral was 0.3 m, the number of turns was 45. Inside the spiral, 6 LED lamps were mounted, providing illumination of the spiral walls of 10 klx and irradiation of 10 W / m². The frequency of illumination and dark phase was 12 hours. The module for bubbling carbon dioxide with a volume of 30 liters was made of glass, dimensions 16 x 38 x 22 cm. It contained a hydraulic pump that provided the supply and circulation of the culture medium into the spiral module. It was also purged with carbon dioxide at a flow rate of 3 liters per hour. After the photobioreactor reached the operating parameters from the bubbling module, a yield of 3 l was taken, and the same volume of fresh dew medium was added. The cultivation temperature is 28 ± 2 °C.

The photobioreactor II was a 50 × 25 × 10 cm glass container with a working thickness of 1 cm, the working volume of the reactor was 1 liter. The top of the photobioreactor was closed by a plastic cover, in which there was a hole for supplying air. Direct sunlight was used as a light source, the culture was sparged with an aquarium compressor, the air flow rate was set at 0.55 liters per liter of culture per minute. The photobioreactor was equipped with an electric heating element to maintain the temperature of the suspension at 45-50 °C. The resulting mass was collected in a centrifuge, the control was count and 3 repetitions were reproduced at each point. Then the optical density was removed and the CFU was calculated, according to the calibration curve, the optical density was matched to the number of viable cells, they were grown to the optical density of viable cells, the correspondence was caught, and a recalculation graph was built.

As a comparison, we used the method of batch cultivation in a 300 ml flask for 10 days at a temperature of 28 ± 2 °C with a photoperiod of 12 h on a standard freshly prepared medium. The culture was illuminated with the same lamps as in continuous cultivation; the illumination on the surface of the suspension was 10 klx.

The density of the biomass in the initial suspension was the same for all variants. Cultural medium: Zarruk's medium was used as a cultural medium [20], the main element of which is sodium bicarbonate (baking soda). Optical density was determined on an Ecoview UV-6100 spectrophotometer (Russia) at a wavelength of 560 nm. The amount of dry biomass was calculated using the coefficient (one OD unit of the culture of spirulina at 560 nm is equivalent to the content of 699 mg of dry biomass in 1 L of suspension) [21].

2.2. Influence of household chemicals at *Spirulina* viability.

The effect of household chemicals, in particular laundry powders and toothpaste, on the growth and development of *Spirulina* was analyzed. To conduct the study, we selected Russian popular detergents by 84% domestic and by 16% foreign production. A live colony of *Spirulina* was placed in pre-numbered glass test tubes, aqueous solutions of laundry powders were added to the same test tubes, the test tubes were placed in a static rack and left for storage in a place with direct sunlight at a temperature of 23-25 °C for 3, 5, 10 days, with periodic stirring of the resulting suspension mass.

For the analysis, we used 50% concentration of working solutions of powders. Checkpoints were considered 3, 5, and 10 days. To check the influence of toothpaste on *Spirulina* viability, 50% solutions of toothpastes were taken: Perioe Pumping, Biorepair, R.O.C.S., Forest Balsam, Sensodyne, Herbals, Levrana, Lacalut, New Pearl, Parodontax. "New Pearl" toothpaste contains: aqua, sorbitol, hydrated silica, sodium lauryl sulfate, xanthan gum, tetrapotassium pyrophosphate, disodium pyrophosphate, sodium monofluorophosphate, sodium saccharin, aroma, sodium benzoate, potassium sorbate, and levrana, formulated as hydrogenated starch hydrolysate (vegetable sorbitol), hydrated silica (silicon dioxide), xylitol, aqua (water), hydrox (hydroxyapatite), arginine (l-arginine), coco-glucoside (from coconut and glucose), citrus sinensis oil (orange essential oil), benzyl alcohol, hondrus crispus (carrageenan), sodium saccharin, *Bidens tripartita* extract (extract streaks), *Rubur idaeus* extract (raspberry extract), *Magnolia officinalis* bark extract (magnolia extract). "Forest Balsam" toothpaste was of the following composition: aqua, silica, sorbitol, hydroxyapatite, sodium lauryl sulfate, cellulose gum, trisodium phosphate, aroma, *Verbena officinalis* extract, *Mentha piperita* leaf extract, sodium monofluorophosphate, *Achillea millefolium* extract, *Chamomilla recutita* flower extract, *Chelidonium majus* extract, glycerin, glycine soja (Soybean) oil, hypericum perforatum flower / leaf / stem extract

(St. John's wort), menthol, phenoxyethanol, phosphoric acid, sodium benzoate, sodium hydroxide, sodium saccharin, *Thymus vulgaris*. The solutions were placed in suspension with *Spirulina*, then the growth of cyanobacteria was monitored on the 3rd, 5th and 10th days of stay with toothpastes. The dynamics of growth and death was determined by CFU/cm³.

3. Results and discussion

3.1. Influence of household chemicals at *Spirulina* viability

Surfactants are one of the major sources of environmental pollution. Surfactants usually include only those substances whose adsorption from solutions already at very low concentrations (tenths and hundredths of %) leads to a sharp decrease in surface tension. Surfactant molecules contain one or more hydrocarbon radicals that make up the oleo- or lipophilic part (it is also the hydrophobic part of the molecule), and one or more polar groups - the hydrophilic part. Oleophilic (hydrophobic) groups weakly interacting with water determine the tendency of the molecule to transition from an aqueous (polar) medium to a hydrocarbon (non-polar) one. Hydrophilic groups, on the contrary, hold the molecule in a polar medium or, if the surfactant molecule is in a hydrocarbon liquid, determine its tendency to transition to a polar medium. Thus, the surface activity of surfactants dissolved in non-polar liquids is due to hydrophilic groups, while those dissolved in water are due to hydrophobic radicals. A significant increase in the level of water pollution with surfactants, including washing powders, shampoos and other detergents should be considered as a special feature of recent decades around the world. *Spirulina*, with its ability to adsorb a wide variety of substances, is also at risk of the negative effects of surfactants. In this work, we tested the effect of one of the widely used surfactants, washing powder, on the survival of *Spirulina*. The effect of household powders on the growth and development of *Spirulina* was checked with using the number of often used popular detergents presented by 84% domestic and by 16% foreign production. Figure 1 shows the composition of a washing powder of one of the common brands.

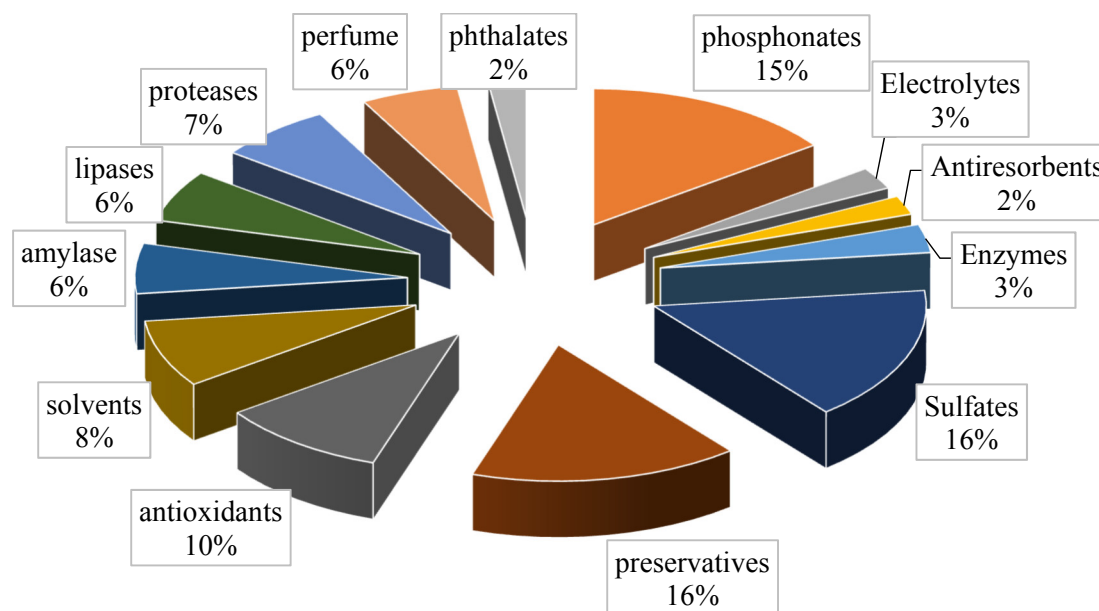


Figure 1. Averaged structure of laundry powder compositions on the Russian market (%)

As can be seen from Fig. 1, in laundry powders sold on the territory of Russia, the most popular component in the compositions are sulphates - 16%, preservatives - 16%, antioxidants 10%, solvents and phosphonates 15%, respectively, it should be noted that for humans one of the highly toxic

substances are phosphonates. They are used in laundry powders to soften water, and in industry as a highly toxic pesticide.

Figure 2 shows that the active growth of *Spirulina* occurs when interacting with the composition of the “Ushasty Nyan” and “BioMio” powders. It is noted that the composition of these powders does not include phosphates and phosphonates, which favorably affected the reproduction of *Spirulina*.

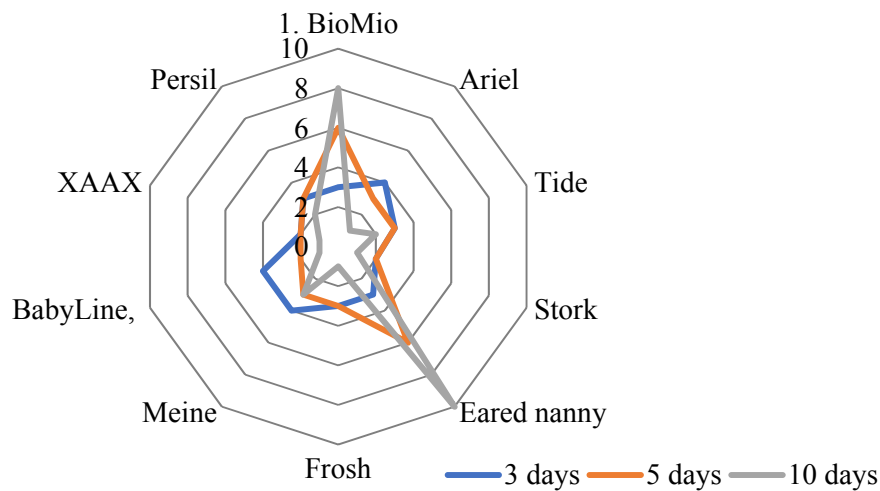


Figure 2. Petal diagram of the growth and death of *Spirulina* under the influence of the chemical compositions of laundry powders

As can be seen from Fig. 3, the composition of toothpastes mostly contains water-soluble compounds that play the role of abrasive components 29%, moisturizers - 23% and thickeners 8%, to a lesser extent functional additives, flavorings, sweeteners, preservatives are included in the toothpastes, and dyes. It should be noted that functional additives can be lured depending on the therapeutic effect of the toothpaste.

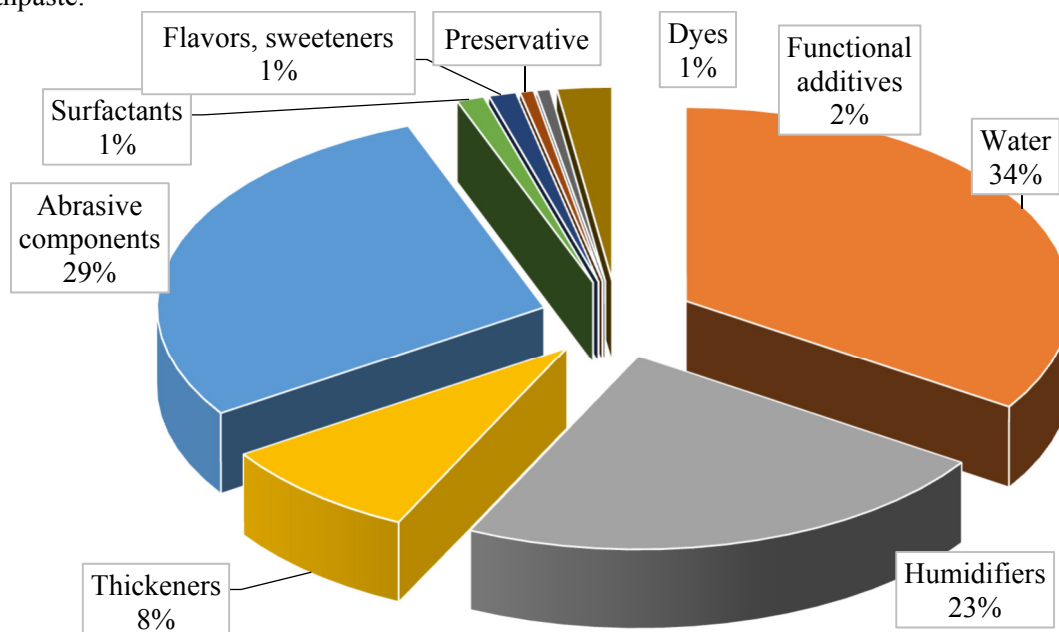


Figure 3. Averaged composition of toothpastes presented on the Russian market (%)

The influence of toothpastes on the growth of *Spirulina* was analyzed, from Fig. 4 it can be seen that the active growth of *Spirulina* in the environment of toothpastes is manifested on the 3rd, 5th and 10th day of the experiment. As seen in the radial diagram in the “New Pearl” toothpaste containing medium provided the greatest growth of *Spirulina* cells. A good result was also shown by the “Forest Balsam” toothpaste containing medium.

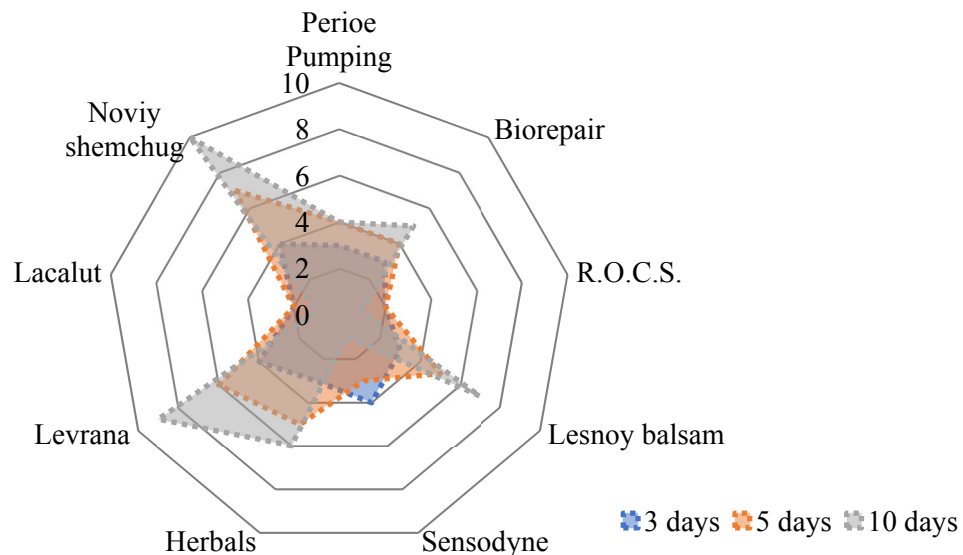


Figure 4. Petal diagram of the activity of *Spirulina* in the environment of toothpastes

It should be noted that with formulations such as R.O.C.S. paste, Sensodyne, Parodontax, Lacalut, *Spirulina* slows down its development and dies on the 10th day of exposition, probably due to the presence of titanium dioxide in the above mentioned toothpastes. Thus, titanium dioxide can be toxic to *Spirulina*, which slows down and suppresses its growth.

3.2. Some issues of *Spirulina* industry

Spirulina valuable qualities have been studied for more than a decade [22]. World production of dried *Spirulina* products is over 12,000 tons per year. It is followed by *Chlorella* spp., *Dunaliella salina*, *A. flosaquae*, *Haematococcus pluvialis*, *C. cohnii* and *Shizochytrium* [23].

Spirulina is a multicellular cyanobacterium that has gained great popularity in the world as a source of valuable protein, vitamins, macro-micronutrients in human nutrition and farm animals (Table 1) [24]. There are few natural sources of *Spirulina*, these are alkaline, mineral-rich, pollution free waters with high pH, namely Lake Chad in central Africa and the Chinese lake Qinghai. Locals in these regions collect spirulina and use it both as a staple food and as an additive to the diet [25], and an additional ingredient in fish, shrimps, and poultry feed.

By a lot of studies, *Spirulina* has been characterized as a wonder drug against number of human diseases [24, 26], e.g., against cancer, diabetes, against allergic rhinitis and asthma, hypertension and hyperlipidemia, heart strokes, anemia, eye diseases, used as immunity booster, as antioxidant, as radioprotective agent, as trace metal supplement, as antiviral, antibacterial and antifungal drug. Also, *Spirulina* can serve as a supplementary cure for many diseases and to cure malnutrition.

Industrial production of *Spirulina* is carried out in two ways - natural production and laboratory one (artificial farms). Natural production takes place in natural ponds based on the natural population of *Spirulina*. Laboratory cultivation of *Spirulina* is carried out in photobiosynthetic cultivators of various designs and volumes. With this method, the intensity and frequency of illumination, temperature and composition of the culture medium are critical.

Table 1. Composition of *Spirulina* [24]

Amino acids	g /100g	Vitamins	mg /100g	Minerals	mg /100g	Carbohydrate	mg /100g	Phytonutrients	g /100g
Leucine	4.94	Vitamin B1	3.5	Iron	100	Glucose	54.4	Cis β -carotene	0.07
Isoleucine	3.20	Vitamin B1	0.32	Copper	1.2	Rhamnose	22.3	Trans β -caroter	0.3
Valine	3.51	Vitamin K	2.2	Calcium	700	Mannose	9.3	Chlorophyll a	1
Tryptophan	0.93	Carotene	140	Zinc	3	Xylose	7	C-phycocyanin (C-PC)	12
Theanine	2.97	Riboflavin	4	Sodium	900	Galactose	3		
Lysine	3.02	Niacin	14	Potassium	1400				
Methionine	1.15	Folic acid	0.01	Phosphorous	800				
Phenylalanine	2.78	Biotin	0.005	Manganese	5				
		Vitamin E	100	Magnesium	400				
Total	22.5		264.035		4309.2		96		13.37

Spirulina is harvested from the growing area and dried at 40 °C for 10 hours until the final water content is below 10%. It is then extracted using Soxhlet (for dried *Spirulina*) and refluxing with sonication (for fresh *Spirulina*) and analyzed for nutrient and bioactive compounds [26, 27].

Figure 5 demonstrates the productivity of the culture of *Spirulina* under two modes of cultivation. In both experiments, active growth of the culture stopped after 8-10 days of cultivation. However, as can be seen from Fig. 5, the productivity of the photobioreactor of continuous cultivation mode was higher.

With the growth of *Spirulina* in a batch mode of cultivation in a carbon-closed system, alkalization of the medium (pH < 11.5) leads to the fact that in the medium carbon is represented only by carbonate ions, which are inaccessible to cells, and the culture growth stops until the pH value decreases and bicarbonate ions appears in the medium. This explains the fluctuations in the density of the culture in the stationary phase and, accordingly, the decrease in the productivity of the photobioreactor [28]. We noted this phenomenon in our experiments also (Fig. 5).

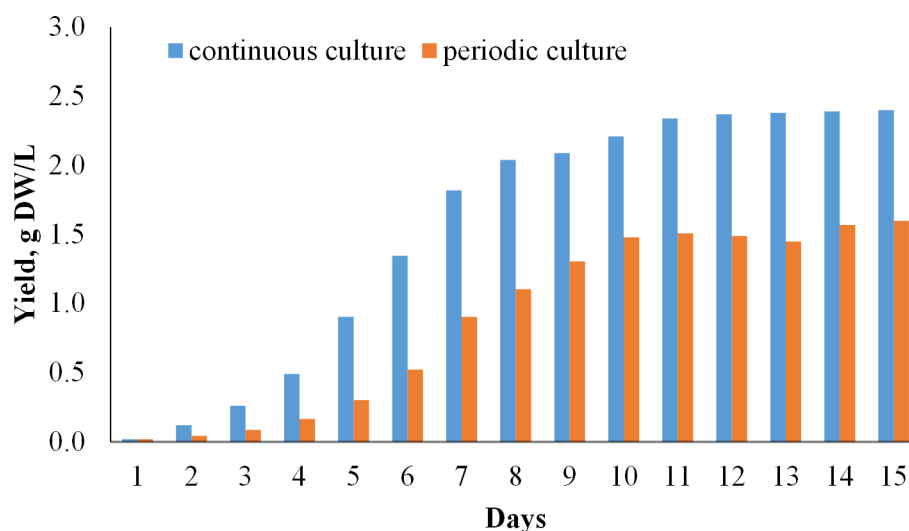


Figure 5. The productivity of *Spirulina* growing in a photobioreactor of continuous and batch cultivation.

4. Conclusions

This work was carried out within the framework of a project for the development of modern technologies for mass cultivation of microalgae for the needs of the food, pharmacological, perfumery, agricultural and other types of industries using a model photosynthetic bioreactor of continuous action. This work presents the results of cultivating *Spirulina* in a continuous phytobioreactor with an additional supply of carbon dioxide, which removes the above-described disadvantages of periodic cultivation. The surprising observation is the positive effect of some laundry detergents on the growth of *Spirulina*. However, the effect depends on the composition of the substances used. The fact that toothpaste has a growth inhibitory effect on *Spirulina's* cells is not surprising, as most of toothpastes are expected to have antibacterial effects.

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