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THE FEATURES OF BIOCHEMICAL INDICES OF STRAIN CHLORELLA VULGARIS IGF № C-111, GROWN IN CLOSED SYSTEM

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ABSTRACT

The article provides optimal concentrations of macro-and micronutrients nutrient medium for microalgae strain SPT S-111 grown as a feed additive for cattle. Utilization of proposed components in the denoted quantity allows getting suspension of chlorella with concentration of 67.2 million cells/ml on the third day of cultivation in the bioreactor gated. It is shown that chlorella grown on our nutritional medium contains is 43.7% of protein, more than 7% of mineral substances, Ca-0.3%, F-0.9%, and less than 5% of fat and carbohydrates in 100 g of dry matter. In 1 g of the chlorella biomass the catalase activity is 0,13 IU/g of tissue, superoxide dismutase - 24.5 IU/g of tissue. The composition of nutrients in chlorella cells indicates that the content of protein, fat and minerals comparable high protein plant products. From the standpoint of the enzyme complex (SOD and catalase) chlorella biomass higher than in other types of plants used for food and medical industries. Grown on this medium chlorella slurry may be used as an additional protein rich provender in the diets to cattle without substantial cost in comparison with synthetic protein additives.

Keywords: chlorella, nutrient medium, biochemical composition

INTRODUCTION

The problem of using the higher and the lower plants as the links of artificial closed ecological systems was fundamentally solved many years ago. Basic research in this direction was made for building closed life-support systems of various objects (space, underwater, underground, etc.). These systems are small models of the terrestrial biosphere. They have a link of photoautotrophic organisms that use the light energy coming from the outside **(Gladyshev, 2007).** The application of closed ecological systems is useful for solving many problems of biotechnology. For example, the use of such systems has found widespread use in the manufacture of unicellular algae Chlorella genus for expanding agricultural animal forage. The choice of this object was due to high yields of algae obtained in mass production cultivation **(Levinskikh, 1987)**. At present, chlorella as a suspension is widely used as high-protein feed supplement in the diets of pigs and poultry **(Bogdanov, 2007)**. But the work associated with the influence of chlorella suspension on physiological parameters livestock fragmentary. Extremely scantily presented information about the diets of livestock, which is included in the chlorella. For inclusion in diets of chlorella suspension of livestock is necessary to select specific conditions of microalgae cultivation. The initial task is the selection of the nutrient medium. Correct choice of medium's composition provides microalgae with all necessary nutrients and ensures rapid growth and biomass increase. The nutrient medium greatly influences on the biochemical composition of chlorella from which in the future depends on the amount of chlorella suspensions to be included in the diet of livestock **(Voskresenskaya, 2009)**.

It is known that for watering agricultural animals necessary to increase the density of the suspension of cells with at least 55 million in 1 ml solution **(Bogdanov, 2007)**. To achieve the economic effect in livestock cells this density must be achieved in a short time and with the lowest concentration of mineral salts used for the preparation of the medium. To achieve the economic effect in livestock cells this density must be achieved in a short time and with the lowest concentration of mineral salts used for the preparation of the nutrient medium. Therefore, the purpose was to selecting the optimal concentration of the culture medium for the cultivation of chlorella in the bioreactor gated with subsequent determination of its chemical composition.

MATERIAL AND METHODS

Chlorella strain IFR S-111 was used as the object of the experiment. Two nutritional media were used – Tamia medium and medium developed in the Biological institute of Saint-Petersburg state university **(Poliak *et al.*, 2008)**. Both of them fulfill the following requirements: provide rapid growth of chlorella cells; chlorella suspension grown in these media is harmless for feeding farmed animals; both cause only a slight increase of main ration cost for farmed animals. Minimal concentrations of macroelements at which there was an intensive capacity of chlorella were found in a series of experiments. Experiments were performed in two bioreactors (parallelepiped glass vessels, 600x150x100 mm). Vessels were divided into two identical compartments of 3 liter volume. 1 concentration of nutritional medium was tested simultaneously (Tab 1). Experiment was carried out in two repetitions. Tamia medium served as control. Each experiment series lasted for three days.

Table 1 Nutritional medium component concentration variants.

Macroelements of nutritional medium	Control	Experiment №1	Experiment №2	Experiment №3	Experiment №4	Experiment №5	Experiment №6
	g/l	g/l	g/l	g/l	g/l	g/l	g/l
KNO_3	5.0	2.5	2.25	2	1.75	1.5	1.25
$MgSO_4*7H_2O$	2.50	1.25	1.13	1	0.9	0.75	0.6
KH_2PO_4	1.25	0.625	0.56	0.5	0.44	0.38	0.3

Initial conditions for microalgae were identical: chlorella was in the phase of active growth. Initial concentrations of chlorella – 28 million cells/ml. Growth temperature – 28°C, illumination 4 thousand lux. Control and experimental samples were taken daily at the same timein order to count chlorella cells in 1 ml of suspension. Cells were counted in Goryaevchamber with Micmed-6 microscope using standard light microscopy techniques.

Upon finding optimal concentrations of macroelements, the following microelements were added, mg/l: $FeSO_4*7H_2O$ – 5; $CaNO_3$ – 10; $Co(NO_3)_2*6H_2O$ – 0.02; $CuSO_4*5H_2O$ – 0.01; $ZnSO_4*7H_2O$ – 0.04; $MnSO_4$ – 1.0; H_3BO_3 – 0.6; $(NH_4)_6Mo_7O_{24}*4H_2O$ – 0.5; EDTA – 5. Biomass increase was counted using Goryaev chamber.

After selection of the optimal nutrient medium was studied biochemical composition of chlorella. The chlorella suspension was grown in the installation of closed type, which consists of two tanks as a parallelepiped placed on a frame made of glass. The culture is illuminated by two fluorescent lamps with a luminous flux of 1400 lumens. Growth temperature – 28°C. It has an automatic heater with thermostat. The slurry is mixed by the water pump capacity of 200 l/ h **(The application for the patent RF № 2013149925 от 07.11.2013)**.

The biochemical composition of chlorella was measured after the third day of it's cultivation. After precipitation and filtration of the suspension different characteristics were determined in the biomass of chlorella: by the method of two definitions of total moisture, crude protein by Kjeldahl's method, crude fiber by Genneberg's and Shtoman's method, crude oil by the method of non-fat residue, crude ash by the weight method, calcium by the complexometric method, phosphorus by the photometric method **(Malinina, 2003)**, the activity of catalase **(Korolyuk *et al.*, 1988)** and superoxide dismutase **(Chevary *et al.*, 1985)**.

All results were subject to statistical analysis in Excel 2007.

RESULTS AND DISCUSSION

Analysis of obtained data has shown that the best biomass increase of chlorella strain IFR S-111 was observed using nutritional medium with mineral salts' concentrations used in experiment 6 (Figure 1 a, b, c, d, i, f).

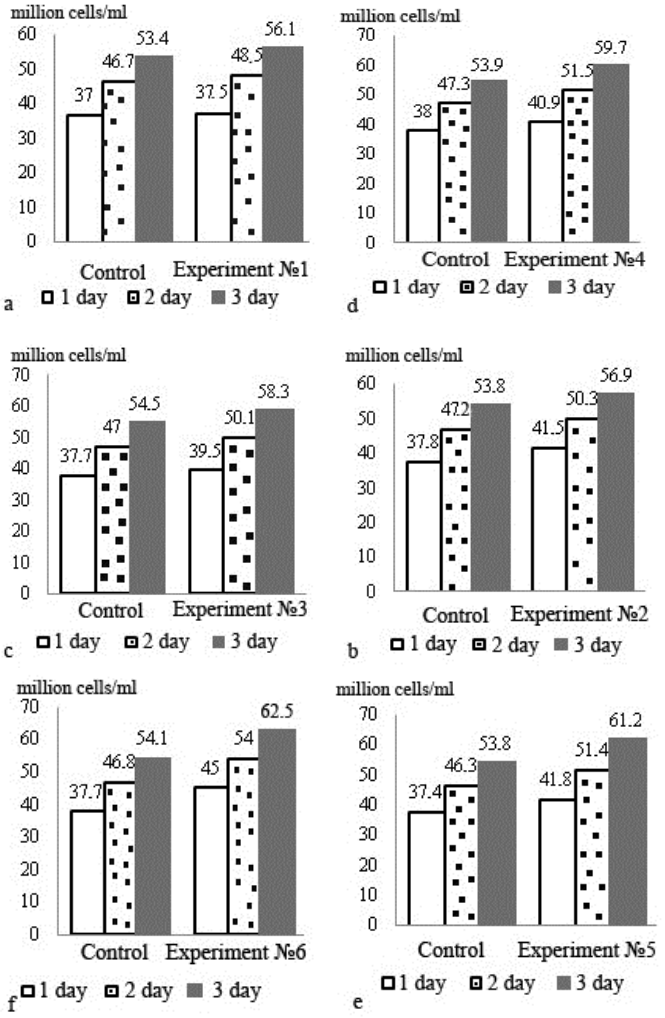

Figure 1 a, b, c, d, i, f Chlorella biomass increase in mediums with various macroelements' concentrations.

Data presented on figure 2 were obtained after adding microelements into the medium with optimal concentrations of macroelements.

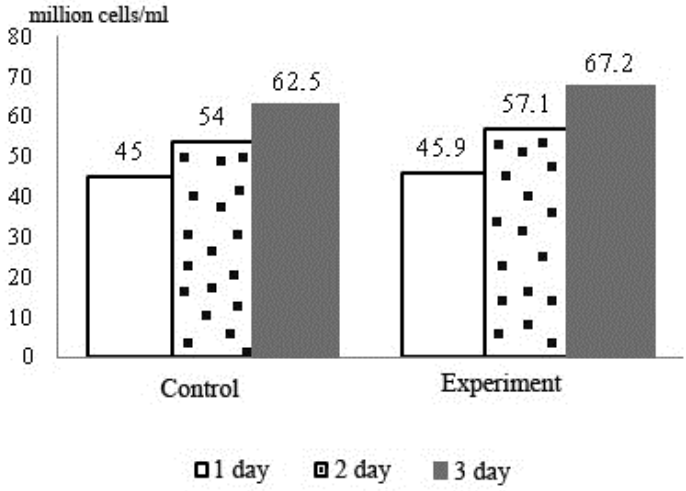

Figure 2 Chlorella biomass increase in media with different compositions of mineral salts.

Therefore, composition of optimal nutritional medium for chlorella strain IFR S-111 found in a series of experiments is as follows. Macroelements (g per liter of water): KNO_3 – 2; $MgSo_4*7H_2O$ – 0.6; KH_2PO_4 – 0.3. Microelements (mg per liter of water): $FeSO_4*7H_2O$ – 5; $CaNO_3$ – 10; $Co(NO_3)_2*6H_2O$ – 0.02; $CuSO_4*5H_2O$ – 0.01; $ZnSO_4*7H_2O$ – 0.04; $MnSO_4$ – 1.0; H_3BO_3 – 0.6; $(NH_4)_6Mo_7O_{24}*4H_2O$ – 0.5; Trilon B – 5. All components included in the obtained chlorella suspension growth medium composition are safe when used for farmed animals feeding.

The analysis of the biochemical composition of the cell suspension of chlorella grown in the experimental bioreactor showed that this single-cell algae is rich in protein (43.7%), as well as mineral components (crude ash – 7.7%; it includes Ca – 0.3%; P – 0.9%), fat and fiber content of less than 5%. As a result, the definition of the enzyme complex isolated from the biomass of chlorella, it was found that in the culture of microalgae the content of catalase is 0.13 UI/g, superoxide dismutase – 24.5 IU/g.

The results indicate that the biochemical composition of chlorella, grown on our medium is not inferior to that grown on different organo-mineral and mineral media **(Bogdanov, 2007; Poliak *et al.*, 2008)**. It should be noted that in our bioreactor cultivated chlorella in their properties are not inferior food items used in the diet of farm animals. The content of protein, fat and minerals in the cells of the single-cell algae, is almost two times higher than the analogic characteristics of grain legumes, which are used to be high-protein components of the diet of animals and humans **(Ivanova, 2012)**. When we compare the activity of catalase and superoxide dismutase in the cells of microalgae with the information about the activity of these enzymes in the leaves of crimson amaranth (*Amaranthus paniculata*) and tobacco leaves, which are used in the pharmaceutical industry as the components for making bio-antioxidant preparations it was revealed that the activity of catalase in the biomass of chlorella was significantly greater than in purple amaranth (*Amaranthus paniculata*). The level of enzyme activity in a purple amaranth ranged from 0.02 to 0.03 IU/g **(Voskresenskaya, 2009)**. The level of SOD (Superoxide Dismutase) activity in the cells of chlorella also exceeds this figure of tobacco leaves. SOD content in tobacco leaves ranges from 22 to 23 IU/g **(Antipina, 2004)**.

Thus grown on this medium chlorella suspension may be used as an additional protein rich fodder for cattle diets without substantial cost in comparison with synthetic protein additives.

CONCLUSION

1. The ratio revealed of macro- and micronutrients of nutritional medium at which the concentration of the chlorella 67,2 million cells/ ml has been reached. Macroelements (g per liter of water): KNO_3 – 2; $MgSo_4*7H_2O$ – 0.6; KH_2PO_4 – 0.3. Microelements (mg per liter of water): $FeSO_4*7H_2O$ – 5; $CaNO_3$ – 10; $Co(NO_3)_2*6H_2O$ – 0.02; $CuSO_4*5H_2O$ – 0.01; $ZnSO_4*7H_2O$ – 0.04; $MnSO_4$ – 1.0; H_3BO_3 – 0.6; $(NH_4)_6Mo_7O_{24}*4H_2O$ – 0.5; Trilon B – 5. The composition and the ratio of all components included in the culture medium for the cultivation of chlorella are safe when they are used in feeding of livestock.

2. The composition of nutrients in the cells chlorella, grown on the this nutrient medium indicates that the content of protein, fat and minerals comparable high protein plant products. From the standpoint of the enzyme complex (SOD and catalase) chlorella biomass higher than in other types of plants used for food and medical industries.

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