ISSUES OF USING MODIFIED A5P-M1 NUTRIENT MEDIUM FOR GROWING CHLORELLA MICROALGAE

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Summary

Chlorella is a microalga with a cell diameter of 2-10 microns that belongs to the family of protococcose green algae. Under normal growing conditions, all chlorella strains form in their biomass from 40-55% protein, up to 35% carbohydrates, 5-10% lipids and 10% minerals.

The paper presents the regularities and requirements for the development of a nutrient medium, on the basis of which, by improving the A5P medium, we have created the A5P-M1 nutrient medium. This medium fully satisfies the needs of chlorella in chemical compounds from organomineral substances such as carbamide, ammophos, K_2SO_4 and Mg_2SO_4.

Experimental work to create a balanced nutrient medium has shown the need to take into account the amount of chemical elements contained in the waters used for growing chlorella. The experiments have shown that in one liter of water in a lake of the Babadurmaz fishery, located 75 km east of Ashgabat, there are 12.4% nitrogen and 22.7% phosphorus for growing one gram of chlorella biomass.

Keywords: chlorella, microalga, biomass, photobioreactor, autospor, strain, lipid.

Chlorella is a microalgae with a cell diameter of 2-10 microns that belongs to the family of protococcose green algae. Chlorella has a single core and grows only through asexual reproduction. The nucleus of the mother cell and the internal environment are divided into several particles, which are covered with a cell wall from the outside and as a result form from 2 to 32 autospores. They increase, gaining nutrients and reaching favorable conditions, and they enter the external environment, tearing the cell membrane and begin to live independently. Usually, the cells of the chlorella microalgae divide (multiply) once a day, but the process of reproduction of their species of intensive growth can occur frequently. The nutritional value and biochemical composition of chlorella, the simple structure of its cells and the high rate of its reproduction, the elasticity of metabolism, as well as properties such as the ability to grow it in artificial conditions, regulation and management of any kind of cultivation parameters turned chlorella into a laboratory biotechnological object at many research institutes (Иерусалимский, 1966; Мельников, Мананкина, 1991; Музаффаров, Таубаев, 1974). The nutritional value and diversity of organic compounds that are formed as a result of its viability opens up the possibility of using chlorella in all branches of the agricultural system. Naturally, chlorella has different strains. They differ from each other in their morphology and environmental conditions of their maintenance. When grown under normal conditions, all chlorella strains form 40-55% proteins, up to 35% carbohydrates, 5-10% lipids and up to 10% minerals in their biomass (Музаффаров, Таубаев, 1974). The protein fraction of the organic substances formed contains all amino acids, including all essential ones (these essential amino acids are not synthesized by them for the human and animal body). Therefore, in terms of quality, the proteins formed by chlorella exceed the proteins of all cultivated forage and food crops. The monosaccharides of the carbohydrate fraction of organic substances formed by chlorella mainly consist of glucose and fructose, and the main polysaccharide is starch. Chlorella contains a small amount of fiber, not exceeding 10%. The lipid fraction of organic substances mainly consists of palmitic, oleic, linoleic and linolenic acids. These fatty acids are dioxide, i.e. unsaturated fatty acids. They make up 80% of all lipids. Unsaturated fatty acids have very high biological and physiological properties. The vitamin complexes formed by chlorella are ahead of all agricultural crops in their quantity and variety of species. The number and amount of vitamins contained in one gram of dry chlorella biomass are shown below (Мельников, Мананкина, 1991):

1. Karotin (provitamin) 100 – 1600 mcg
2. B1 vitamin – 2-18 mcg
3. B2 vitamin – 21-28 mcg
4. Vitamin B2 – 0.025-0.1 mcg
5. S vitamin – 1300-5000 mcg
6. K vitamin – 6 mcg
7. PP vitamin – 110-180 mcg
8. E vitamin – 10-350 mcg
9. D provitamin – 1000 mcg
10. Pantothenic acid – 12-17 mcg
11. Folic acid – 485 mcg
12. Viotin – 0.1 mcg
13. Leucovorin – 22 mcg

In the process of growth and development, chlorella, as a result of its vital efficiency, produces intermediate and final metabolic products, many different amino acids, enzymes, vitamins, organic acids such as citric, malic, succinic and formic acids. Such a rich composition of chlorella turns it into one of the main objects of biotechnology. In different countries, high rates have been obtained with the use of chlorella as a feed additive for farm animals. Moreover, on the basis of different biotechnological methods, it is possible to obtain biofuels of different quality.

Under artificial conditions, chlorella is grown in specially manufactured glass-tube photobioreactor plants. The main requirements for a photobioreactor: it must have the ability to freely perform the gas exchange process, ensure a uniform incidence of sunlight (illumination) on the photosynthetic surface and the ability to regulate and control such growing parameters as the temperature of the liquid mineral nutrient medium, the amount of carbon dioxide (CO_2) supplied, the possibility of unhindered addition of minerals that make up the nutrient medium, the circulation of chlorella with the nutrient medium inside the photobioreactor at a certain rate.

When growing chlorella in artificial conditions, environmental conditions such as the temperature and pH of the nutrient medium, the amount of CO_2, and the power of light incident on the surface of the photobioreactor is also the priority condition.

When growing chlorella artificially, one of the most basic conditions is the production of a nutrient medium for its cultivation. This medium consists of aqueous solutions of mineral or organomineral substances. Since the direction of chlorella biosynthesis is focused on the formation of proteins, it needs a large amount of nitrogen (nitrogen is part of amino acids, protein monomers). For its normal growth, macronutrients such as phosphorus, potassium, magnesium, sulfur and calcium are needed in a certain amount. The absence or shortage of these macronutrients leads to the destruction of the processes associated with the growth and reproduction of chlorella cells, and they die. For the normal growth and reproduction of chlorella cells, along with macronutrients, a specially developed complex of trace elements is also needed. They accelerate and regulate such processes of chlorella cultivation as growth, reproduction and biomass formation. The nutrient medium for chlorella is created based on the following patterns and requirements:

a) the minerals included in the nutrient medium should be close to their content in the chlorella biomass (balanced medium);

b) during the entire time of chlorella cultivation, the pH of the nutrient medium should be maintained uniform (approximately 7);

c) mineral salts should not be in the form of precipitation;

d) in the created nutrient medium, chlorella should grow and multiply evenly.

Based on these patterns, we improved the balanced A5P medium (Упитис, 1983) and created the A5P-M1 nutrient medium on its basis (Сейтгельдыев, Упитис и др., 1992). This nutrient medium consists of organomineral substances such as carbamide, ammophos, K_2SO_4, Mg_2SO_4 and fully satisfies the needs of chlorella in chemical compounds.

The environmental conditions of growing each of the different chlorella strains have certain parameters (temperature, chemical composition of the nutrient medium, lighting, etc.). Under these conditions, all strains form, especially in large quantities, proteins (up to 55%). If, by changing environmental conditions, you create a stressful state for chlorella, then its strains form various amounts of organic compounds. As a result, the concept of lipid-forming chlorella strains arises.

The waters intended for scientific research are taken from three lakes with approximate dimensions of 150x200 square meters, located 10 meters from each other in the Babadurmaz area, located 75 km from the city of Ashgabat. The chemical composition of these waters was analyzed using a photoelectric calorimeter. We thought that this analysis would almost completely clarify this problem, because, firstly, the amount of salts freely lying on the bottom of lakes is considered relatively small, and secondly, when growing fish artificially, some of the substances from fish feed dissolving in water can pass into its composition.

The 1st table shows the results of analyses of lake waters for the maintenance of fish in the Babadurmaz area.

Table 1

Results of analyses of lake waters for the maintenance of fish in the Babadurmaz area

№	NO_3 mg/l	NH_4 mg/l	NO_2 mg/l	Orthophosphates, mg/l	pH
I	4,000	11,000	2,400	12,400	7,432
II	4,880	3,500	2,260	3,600	7,544
III	4,880	8,680	2,260	9,040	7,648

When growing chlorella in a photobioreactor, an average of 82 mg of nitrogen and 17.6 mg of phosphorus are needed to form 1 g/l of dry biomass (Мельников, Мананкина, 1991; Музаффаров, Таубаев, 1974). The main goal was to determine the content of these elements in the composition of water taken for analysis using a Photoelectric calorimeter. If you determine the amount of these elements in the water, then it is possible to determine which part of all the necessary nitrogen and phosphorus are already ready for use. However, chlorella microalgae cannot absorb all forms of nitrogen and phosphorus, it can only absorb nitrogen nitrates, ammonium ion salts and nitrite forms of nitrogen. And it assimilates its orthophosphate (phosphoric acid salts) forms from phosphorus. Therefore, during the chemical analysis, attention was paid mainly to the content of these compounds. Since the fish were launched to the first lake 2 days earlier than the rest, chemical calculations were carried out based on the indicators of this lake.

Calculations have shown that in the composition of the water of this lake there are forms of nitrogen assimilated by chlorella in the amount of 10.2 mg/l and phosphorus (PO_4) 12.4 mg/l. From here, it is possible to determine the phosphorus content in 1 liter of water, which can be absorbed by chlorella:

$$12.4 \times 0.326 = 4 \text{ mg/l}$$

Next, we determined how many percent of the elements nitrogen and phosphorus present in the lake water are from their total amount necessary for the synthesis of 1 g/ l of dry chlorella biomass. Calculations have shown that for the synthesis of 1 g/l of dry chlorella biomass, the required amount of nitrogen is on average 82 mg/l. And the lake water contains 10.2 mg/l of nitrogen. From here, it is possible to calculate how much of the required nitrogen (N) is in the composition of lake water:

$$10.2 \times 100: 82=12.4\% \text{ N}$$

To synthesize 1 g/l of dry chlorella biomass, the required amount of phosphorus is 17.6 mg/l on average. Then it is possible to determine what part of the necessary phosphorus (P) is in the composition of lake water: 4 x 100:17,6= 22,7% P. With the help of these calculations, it is possible to determine in what quantity and form nitrogen and phosphorus compounds are added. So, we add the missing amount of nitrogen and phosphorus from ammophos and fiber (urea). When the missing amount of phosphorus is added from ammophos, it must be borne in mind that there is a certain part of nitrogen in the composition of ammophos. Therefore, this amount is taken into account when adding urea to the nutrient solution (medium).

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