Selection of the Composition of the Fertilizer and Optimal Factors for the Cultivation of Green Microalgae on Phosphorus-Containing Waste in the South of Kazakhstan

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Keywords: Green microalgae, Phosphorus-containing waste, Biofertilizers, Chlorella vulgaris.

Abstract: Modern agricultural production technologies based on the widespread use of pesticides and mineral fertilizers have largely solved the problem of providing the population with food, and at the same time have generated multiple ecological, medical and environmental problems, problems of environmentally friendly and biologically valuable food products, land rehabilitation, restoration of their fertility, etc. Therefore, the emergence of new classes of pesticides with different mechanisms of action, high selectivity and low toxicity for warm-blooded animals is very modern. Currently, the development and application of new plant protection products that are not toxic to humans and animals is of global importance. Priority is given to research aimed at creating plant protection products based on microorganisms and their metabolites, as well as the search for plant substances with potential pesticide activity. In this regard, the question arose of finding new safe fertilizers that can also be economically profitable for production on an industrial scale. One of the current trends in this industry is the use of green microalgae.

1 INTRODUCTION

The Republic of Kazakhstan is one of the major agro-industrial powers of the world, which supplies grain and legumes, fruits, vegetables to 70 countries. In the long-term development plan of Kazakhstan's economy, it is planned to expand foreign sales markets from $ 3.5 billion in 2021 to $ 9.9 billion in 2030. The structure of agricultural exports is as follows: wheat - 5.7 million tons or 37.5%; barley - 0.8 million tons; flax seeds -333.6 thousand tons; flour -1.5 million tons, sunflower oil -97 thousand tons, cotton fiber - 63 thousand tons (SHapiro, 2003). It should be especially noted that Kazakhstan is among the top ten countries producing environmentally friendly organic products.

However, intensive use of soil resources leads to a decrease in the content of nutrients in it. According to Kazakh scientists, the lack of organic fertilizers and an imbalance in the use of mineral fertilizers led to a shortage of nitrogen, phosphorus, potassium and a number of trace elements in the soil. In these conditions, it is necessary to change the existing system of land use and agricultural technology of cultivation of agricultural crops.

Currently, about 180 million hectares are covered by technogenic desertification in all natural zones of Kazakhstan. According to official data, almost all arable soils of Kazakhstan have lost up to 20-30% of humus, and 12 million hectares are exposed to wind, 5 million hectares of water and 500 thousand hectares of irrigation erosion (Bajshanova, Kedel'baev, 2016). Moreover, as a result of haphazard grazing, 63 million hectares of pastures are in various stages of degradation.

Biotechnologies in agriculture, especially in land husbandry, offer various ways to increase the efficiency of fertilizers. The problem of increasing the efficiency of land husbandry does not lose its

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relevance. One of these directions is the introduction of bacteria into the soil that can effectively enrich it with bioavailable nitrogen and phosphorus. For example, in some studies, nitrogen fixers and phosphate-mobilizing microorganisms, ammonium nitrate, phosphorous flour in conventional and nanoscale form are introduced into the soil (Kobegenova, SHakenova, 2017). Ammonium nitrate is a widely used source of nitrogen, and phosphorite flour is an effective and safe mineral additive with a long-term effect. On the territory of the CIS, the preparation mizorin is often used with a strain of *Arthrobacter mysorens*, which has a positive effect on many agricultural crops (Aleshchenkova, Safronova, Mel'nikova, Esenbaeva, Ten, 2015; Kovalevskaya, SHaravin, Bessonova, 2018). The authors noted that when conducting a comparative analysis using ammonifying microorganisms, the phases of ontogenesis amounted to about 19.0-23.5 million/g, while when adding ammonium nitrate and myosin, the number of microorganisms increased to 25.5-41.0 million/g. A similar study was conducted with the bio-preparation "BioAzoPhosphite" on the territory of Kazakhstan, where wheat grains were treated with this preparation with the calculation of 2 L/t (Zavalin, 2015). As part of this biological product, there are also nitrogen–fixing and phosphorimobilizing microorganisms, which, according to research results, have shown that "BioAzoPhosphite" during the pre-sowing treatment of flax and barley seeds, with a consumption rate of 1.5-2.0 l/t, increases the laboratory germination of seeds, and moderately increases the density of plant seedlings, and also suppresses the infection of seeds with mold fungi.

A biological alternative to mineral nitrogen fertilizers in agriculture is the use of fertilizers based on nitrogen-fixing organisms (Moskvichev, Balashov, Pyatibratov, 2009). Microbial fertilizers have a number of advantages over mineral fertilizers:

1) they are harmless to humans, animals, birds and insects
2) improve soil fertility
3) cheap to manufacture
4) the production of biological fertilizers and their use does not harm the environment, since the components of biological products do not accumulate in ecosystems and are easily disposed of.

The use of microorganisms and substances derived from them has great advantages in comparison with plant and animal cells (Mertasov, Babak, ZHakupov, 2018). For the production of bacterial fertilizers, microorganisms are cultivated in bioreactors, where the necessary conditions are created, such as nutrient medium, aeration, temperature, pH, selection of metabolic products, absence of contamination. In bioreactors, strains of microorganisms use nutrient media and synthesize the necessary industrial products or microbial biomass. The resulting biomass is separated and mixed with an adsorbent, if necessary, soil, peat, vermiculite, sawdust, etc. are used as the basis for immobilization (Rusanova, 2001).

In the modern agro-industrial complex, a wide range of biofertilizers are used, which have various properties such as nitrogen fixation, plant growth stimulation, suppression of the growth of phytopathogenic microflora.

For example, a biopreparation based on PGPR-bacteria, PGPB, which is inoculated directly on the plant and has a growth-stimulating property, has become widespread. Scientists note that this biological preparation activates nitrogen fixation in the rhizosphere and increases the overall microbiological activity in the root system (Tepper, 2004). Nitrogen-fixing bacteria occupy a special place in the cultivation of sorghum, barley and winter wheat. Thus, when nitrogen-fixing microorganisms were added to the soil, sorghum productivity increased by 15-20%, while nitrogen fixation increased by 20-50% (Dyatlova, 2001). In the variant of studies with winter wheat, the highest yield was shown by the experimental variant with a combination of nitrogen fertilizers and nitrogen-fixing bacteria (Hotyanovich, 1995).

We will give a brief description of popular biological fertilizers widely used in agriculture.

Nitragin. The birthplace of this biofertilizer is Germany, the production of Nitragin began in 1896, the authors of the development are F.Nobbe and L.Giltner. Nitragin is most often used on soils where legumes are supposed to be cultivated. In the experiments of Mishustin E. and Bernard V. (Zavalin, 2005), various types of soils of the European part of Russia were used. According to the results, it was found that the yield increased in all soils. Nitragin showed the best result on acidic soils. The high efficacy of this preparation is associated with the presence of *Rhizobium* and *Bradirhizobium strains*, which, when present in the environment of legume roots, show high nitrogen fixation productivity.

On the other hand, with frequent sowing of legumes without changing lands, there is a decrease in crop productivity. This is due to the accumulation of phytopathogenic forms of microorganisms in the soil, such as pathogenic and saprophytic fungi. In the course of studies of these fungi, a strain of *Gliocladium zaleskii 278* was discovered, which has phytotoxic properties (Emcev, 2014).

An effective solution to increase the yield of agricultural plants is the use of a complex of legumes, nitrogen-fixing bacteria and a phosphate-immobilizing fungus. Also, to improve the properties of *Bradyrhizobium japonicum*, modification of the strain with lectin is proposed (Gorban', 2007).

One of the necessary conditions for the use of microbial biologics is soil moisture, therefore, in arid zones, due to low precipitation and humidity, the effectiveness of microbial biologics may decrease (Labutova, 2007). In the study of Kazakhstani scientists, nitragin showed the best result in the chernozems of Northern Kazakhstan (Sytnikov, 2007).

Phosphorobactorin is a microbial preparation that contains the spore-forming bacterium *Bacillus megaterium war. phosphaticum.* After cultivation in a bioreactor, the finished biomass is mixed with white clay. The finished biological product can be stored for a year. This preparation is used in the treatment of plant seeds, getting into the soil *Bacillus megaterium war. phosphaticum* decomposes organic phosphorus into a form accessible to plants (Biederbeck, Bouman, Campbell, 1996).

Azotobacterin. Azotobacterin is one of the first biofertilizers used since the USSR, which contains a culture of *Azotobacter chroococcum*. It was found that Azotobacter not only fixes nitrogen necessary for plant development, but also produces organic compounds such as vitamins and growth stimulants. Moreover, azotobacter has a fungistatic property and is effective for a wide range of phytopathogenic mold fungi. The main disadvantage of azotobacterin is its inefficiency for field crops, it shows itself well only in cultivated soils. For example, this biological product shows a high yield of up to 20-30% on soils previously fertilized with manure. With additional application of azotobacterin, favorable conditions are created for the root system of plants (Karagujshieva, 1981). In the industrial production of azotobacterine, only active strains of azotobacter are used, which not only fixes nitrogen, but also partially releases it into the nutrient medium. In addition, when using a biological product, it is necessary to take into account the properties of the soil (Lovley, 2003). Studies of azotobacter have shown that in combination with other microorganisms, the effect on plant development and yield increases. For example, a pure strain of azotobacter releases 173 mg of the hormone auxin into the medium, while when *Bacillus mycoides* is added, this figure increases to 220 mg (A Citizen's Guide to Soil Washing, 2004). Numerous studies show that mixed cultures are much more effective, have increased nitrogenase activity and are able to create syntrophic associations (Sidorenko, Borisenko, Van'kova, Vojno, 2005).

Studies to determine the optimal ratios of microorganisms have shown that some microorganisms suppress the properties of azotobacter. For example, when *Bacillus subtilis* was added, the number of *Azotobacter chroococcum* cells decreased by 2-5 times (Umarov, 2001). It was found that this type of bacteria uses root secretions of plants as a food source, producing compounds that inhibit the growth of azotobacter (Kisten', Kudish, Bega, Carenko, 2006). One of the limiting factors for the development of azotobacter culture is the lack of a carbon source in the soil, that is, when applying azotobacter, an addition in the form of organic fertilizers is necessary (N'yuton, 2002).

Azotobacterin compared to the above-described nitragin proved to be more effective in arid areas. When using azotobacterin, growth substances in the root system are activated and germinate through the drying horizon and the plant seedling is better supplied with moisture (Safin, 2007). On the other hand, compared with nitragin, azotobacterin is ineffective on acidic soils. To increase the effectiveness of the biological product, additional liming of the soil is necessary. Specially for acidic soils, a biological preparation based on fossil limestone and azotobacter - AZ-Kalk has been developed, which corrects the level of soil acidity and increases soil fertility.

Studies have also been conducted on the use of azotobacter with the addition of micronutrients (Kurdish, Bega, Gordienko, Dyrenkov, 2008). According to the research results, it was found that the crop yield increases by 2.4 c/ha. When studying another species of azotobacter *Azotobacter vinelandii*, results were obtained that showed that this species stimulates seed germination to varying degrees (Antip'eva, SHerstoboeva, SHerstoboev, 2001). Most likely, this is due to the different susceptibility of plant seeds to Azotobacter culture.

In agriculture, biologics based on free-living oligonitrophils, such as *Bacillus polymyxa*, are popular, in addition to nitrogen fixation properties, they have static properties to a wide range of phytopathogenic fungi (Skvorcova, 1998).

After research on the creation of a symbiosis between nitrogen-fixing microorganisms and non-leguminous plants, a new direction of research began to develop, which was based on the nitrogenase activity of microorganisms. These bacteria are potentially useful, but their ability to create complexes has not yet been fully studied (Kopylov, 2007). On the basis of these studies, biologics

Agrophil, Agrofor, Azorizin, Mizorin, Rhizoagrin, Flavobacterin and others were created (Bekker, 1988). The biopreparation Extrasol was also developed, which contains a whole complex of soil and rhizosphere microorganisms such as *Arthrobacter mysorens* K., *Flavobacterium sp L* 20, *Agrobacterium radiobacter* 204, *Azomonas agolis* 12, *Bacillus subtilis* 4-13, *Pseudomonas fluorescens* 2137, *Azospirillum lipoferum* 137. According to the type of exposure, three types of biofertilizers are produced, these are extrasol-90 – fungicidal action, extrasol-09 – stimulating action and extrasol-55 – fungicidal-stimulating action (Furina, 2007). Work was also carried out with the *Azospirillum brasilense* strain 104. When inoculated on spring wheat grains, this strain increased nitrogen fixation by 2.2 times, and chlorophyll a and b in leaves increased by 86.5%. It was also noted that azospirilla reduce the spread of root rot by 1.7-1.8 times. According to the results of studies with azospirilla, it was found that wheat yield increased by 26.4% in three years.

In addition to the effect on agricultural crops, azospirilla was used as a growth stimulant for edible mushrooms (Gromov, 1989). With this application, the mycelial biomass of fungi increased by 30-35%, moreover, the cultivation time of these fungi was reduced.

A Bioplant-K biopreparation based on a strain of a bacterium of the genus *Klebsiella* (*K.planticola*, strain TLCA-91) is known (SHulyakovskaya, Sasova, 2012). This preparation is recommended as a bacterial fertilizer for vegetable crops. The beneficial effect of Bioplant-K on plants is determined by the presence of nitrogen-fixing, vitamin-producing microorganisms in the preparation. With the use of this preparation, the yield of cucumbers increased by 21-23%, tomatoes and pumpkins – by 31%, potatoes – by 21%.

The use of biological preparations based on a complex of microorganisms is promising. For example, joint inoculation of *Azospirillum lipoferum* and *Sinorhizobium meliloti* increased alfalfa yield several times (Novikova, 2019). And inoculation of seeds with a complex of *Azospirillum* and *Rhizobium* increased the number of nodules in legumes by 2-3 times, contributed to an increase in nitrogen fixation and improved mineral nutrition. The formation of nodules on the root system was also noted earlier (Bhattacharjee, Dey, 2014).

In modern agriculture, the joint use of associative nitrogen-fixing bacteria and mycorrhizal fungi is also promising. In this case, a symbiosis appears in which the bacterium supplies all partners with nitrogen, the mushroom-orderly kills pathogenic microorganisms and helps the plant to absorb water and minerals, and the plant provides microorganisms and fungi with organic substances (Smirnova, Karimova, SHnejder, 2016). An example of such a symbiosis is an artificial symbiosis: sorghum, flavobacteria and the fungus *Glomus fasciolatum*, while the sorghum yield increases by more than 50%.

According to the estimates of the International Organization, it was shown that about 70% of the land area of the globe is represented by unproductive lands, the productivity of which is limited by soil-climatic, relief or economic conditions. With the development of agro-industry and the need to increase productivity, the overall pressure on soil productivity increases. According to monitoring data, it was revealed that over the past half century, the area of these soils has decreased to 300 million.ha, and the lost carbon was about 38 billion tons. According to L.N. Shulyakovskaya, it is difficult to solve this issue without the involvement of naturally unproductive and degraded lands (Smirnova, Karimova, SHnejder, 2016). One of the problems of reducing the biological activity of soils is non-degradable crop residues, which reduce the yield of agricultural crops. Studies by Novikova I.I. show that phytopathogens have been found in these residues, which, despite a large amount of time for recovery, remain in the soil and occupy most of the soil microflora, about 75% (Krutilo, 2014).

In the agro-industry, enzymatic preparations such as the American biologics AG-zyme and HC-zyme have been widely used in recent years. Analogous preparations are produced by Ukraine, which are called – Dorzin, Agrozin and Oxyzin.

Microbiological preparations such as nitragin, rhizotorphin, azotobacterin, phosphobacterin are widely used. Numerous studies show its high efficiency in increasing soil fertility. These biofertilizers not only increase soil fertility, but are also economically beneficial as there is no need for a large amount of mineral fertilizers. This reduces the load on the soil, and also reduces the recovery time of soil resources (Zharkova, Manylova, Bykov, 2019).

According to Smirnova I.P., one of the most popular biological preparations are preparations based on the microscopic fungus trichoderma. In agriculture, it is known as Glyocladin, which is an analogue of the preparation Trichodermin. The microscopic fungus trichoderma has an overwhelming effect on phytopathogens and is their direct competitors for plant residues.

Biopreparations based on nodule bacteria strains have been created, which have the ability to increase the yield of green mass of agricultural crops, such as

alfalfa and sainfoin by 70-80%. Such preparations are "Rhizobophyte" (Moskvichev, Karpova, Egorov, 2018), which contains a vermiculite base and 7.5-8 billioncl/g of tuberous bacteria, and "Rhizotorphin", which contains a peat base of 10-15 billion tons. cells of nodule bacteria.

The market of biological products is annually replenished with new types of products adapted to different types of soils and weather and climatic conditions of the area. Priority in the development of biologics of ecological action belongs to Russia and the USA, while the basis of biologics are microorganisms of various taxonomic groups. The scheme of development of biological products consists of the stages of isolation of dominant groups of microorganisms, selection of active strains, study of oxidative capacity, assessment of their impact on the environment, efficiency of use in *situ and* ex situ conditions. However, despite the diversity of the biologics market, most of them are confined to specific environmental parameters and are ineffective in the arid climate of Kazakhstan. The peculiarities of the development and production of biological products for the south of Kazakhstan are associated with a shortage, a high level of ultraviolet irradiation and salinity in water and soil. These moments become key obstacles when trying to use well-known foreign and domestic biological products to solve local environmental and agricultural problems in the south of Kazakhstan.

2 MATERIALS AND METHODS

The objects of research were phosphorus-containing waste from the former plant for the production of phosphorus fertilizers in Shymkent: slags, slurries and phosphorus-containing wastewater. Currently, the waste is stored on the territory of Kainar LLP and occupies an area of about 16.6 hectares (Figure 1).

A. Location of phosphorus-containing waste in the border of Shymkent, B. Kainar LLP, C. phosphorus-containing sludge, D. Phosphorus-containing wastewater

Figure 1: Storage location of phosphorus-containing waste in Shymkent (coordinates of the sampling point: 42.2703059N, 69.7182539.276E).

Kainar LLP is the main producer and supplier of phosphorus fertilizers in the south of Kazakhstan. The manufactured products of KAINAR — mineral fertilizer "superphosphate" — granular phosphorus fertilizer containing 15% of the digestible P2O5, 2-4% K2O. Simple superphosphate is used for all agricultural crops and on all soils. Approximately 75-80% of the waste is granular. The appearance of the site with phosphorus waste seems uneven, their volume is approximately 0.5 million tons. If we talk about the general environmental situation, the area around the waste is polluted with household and technological waste. The color of granular and dense waste of phosphorus production varies from white to black, the acidity of the medium ranges from 6.0-7.2. In order to prevent the ignition of phosphorus-containing sludge under the influence of oxygen in the air, they are kept in a wet state. Phosphorus-containing wastewater is formed from fire-fighting waters and storm-sedimentary waters, which, passing through the thickness of sludge and slag, are collected in various technical reservoirs (Figure 1, d).

Sampling technique. Water samples were taken according to the methodological guidelines **(**GOST 18826-73, 4388-72, 18293-72, 18309-72, 4245-72, 3351-74, 4979-49, 4151-72 and 18293-73**)** (Figure 2).

Figure 2: The process of collecting phosphorus-containing wastewater at Kainar LLP.

The elementary composition of the waste was determined using ICP, volume-weight parameters and structure by means of an electron scanning microscope of the brand JSM 649LV JEOL (Japan). The microanalysis was carried out using the INCA Energy 35O OXFORD Instruments microdisperse system (UK), the analysis on the territory of HKL Basis was implemented using a polycrystalline analysis system.

Microscopy of the preparations was carried out using a Biomed-5 microscope with a 10×; 40×; 100× lens using immersion oil (GOST 28489-90). The study of the water sample was carried out using the drug "crushed drop". At the same time, fat-free cover and glass slides of 75×25 mm and 18×18 mm, pipettes of 1 and 2 mm, respectively, were used. To study mobile hydrobionts, a weak KI solution was used to fix them.

The taxonomic analysis was carried out on the basis of determinants. A camera (*Samsung Digimax* S600) was used for video recording of the studied objects.

Microorganisms were grown on appropriate liquid and solid elective media. In microbiological culture, the methods of Koch, Novogrudsky, "exhaustive culture method", and marginal tenfold dilutions were used. Pure cultures were isolated on the mown nutrient agar. Nutrient media and microbiological dishes were sterilized according to the conditions in a bacteriological autoclave (SPGA-100-1-NN). The cultivation of microorganisms was carried out in a thermostat with a programmable temperature (TC 1/80). All microbiological cultures were carried out in a pre-prepared microbiological box. As elective media were used (GOST R 51758-2001):

Czapek medium for micromycetes of the following composition, g/l: sucrose – 30.0 g; KH_2PO_4 – 1.0 g; $MgSO_4 \times 7H_2O$ – 0.5 g; $NaNO_3$ – 3.0 g; KCl – 0.5 g; $FeSO_4 \times 7H_2O$-0.01 g; agar – 20 g.

Fish peptone agar for heterotrophic bacteria of the following composition, g/l: pancreatic sprat hydrolysate – 10.05g; NaCl – 4.95g.

Ashby medium for nitrogen-fixing microorganisms of the following composition (g/l): $C_{12}H_{22}O_{11}$ – 2.0g; K_2HPO_4 – 0.2g; $MgSO_4 \times 7H_2O$– 0.2g; NaCl – 0.2g; K_2SO_4 – 0.1g; $FeSO_4$ – 2 drops 1% solution; $CaCO_3$ – 0.5g; agar – 20.0g.

Tamiya medium: KNO_3-5.0; $MgSO_4 \times 7H_2O$-2.5; KH_2PO_4-1.25; $FeSO_4 \times 7H_2O$-0.003; Trace element solution – 1ml, EDTA-0.037

Composition of the Pratt medium (g/l): KNO3 - 0.10 MgSO4*7H2O - 0.01 K2HPO4 - 0.01 FeCl3*6H2O - 0.001 agar-agar - 12 g

ITA medium, g/l: phosphorus–containing slags - 10; KNO3 - 0.10 MgSO4*7H2O - 0.01.

In the control version, tap water was used to water the test plants. In the experimental versions, various percentage solutions of phosphorus-containing waste and algae were used .

The taxonomic affiliation of bacteria was carried out according to the "Determinant of the Bergi bacterium", micromycetes according to the "Determinant of pathogenic and conditionally pathogenic fungi". The morphology of micromycetes was determined by colonies on Petri dishes. When describing colonies, the shape, cross-section, edges, texture, color, and pigment diffusion on agar were taken into account.

When preparing the nutrient medium, scales of the Scout-Pro brand (GOST 24104-2001) were used, and an autoclave of the SPGA-100-1-NN No. 141 brand was used during sterilization. To determine the bacterial titer, the sample obtained after quartering, with a volume of 1 g, was stirred in 100 ml of water on a shaker for 30 minutes. The resulting suspension was diluted in a nutrient medium by 10-fold dilution. 1 ml of the sample suspension was cultured on a nutrient medium. The cultivation of microorganisms took place at 25 °C for 7 days.

Laboratory utensils and nutrient media were sterilized in an autoclave (SPGA-100-1-NN No. 141). The colonies were counted after 72 hours.

Algae cultivation was carried out in a sterile box on solid nutrient media of Myers and Pratt. Incubation of the culture produced on a liquid nutrient medium was carried out on light racks (No. GS-1/80 SPUTU 9452-002-00141798-97) at a temperature regime +23+25 °C. The cumulative culture of microalgae was grown on light racks in plastic dishes, with a volume of 5 liters, at a temperature of 23-25 °C with constant aeration. The content of Pb^{2+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} ions in the aqueous medium was determined on the STA –1 complex, using the method of inversion voltammetry and atomic adsorption method on the AAS 1 spectrophotometer. Ions of chlorides, sulfates, nitrates and nitrites by the photocolorimetric method on the KFK-3-01-ZOMZ photometer and the ionometric method on the I-500 ionomer.

The preparation of percentage suspensions was carried out according to GOST 4517-87. 5.0%, 10.0%, 15.0% and 20.0% slag and sludge suspensions were prepared, in which microalgae strains were cultivated for 7 days. The original and modified Pratt medium with the addition of phosphorus-containing waste was used in the research (10 g of phosphorus-containing waste was added instead of 10 g of potassium phosphoric acid, double-substituted according to the traditional recipe). Cultivation was carried out for 7 days at a temperature of +23 °C with a 12-hour light day.

For sowing test plants, disposable plastic cups with 50 g of sterile expanded vermiculite were used, where calibrated seeds of test plants inoculated with chlorella were planted. To maintain moisture exchange, the glasses were tightly closed with a film.

Morphometric data: the length of plants, stems and roots; mass indicators were measured with a ruler and on analytical scales. The number of leaves on seedlings was calculated visually.

The qualitative composition of lipid extracts of microalgae was carried out by thin-layer chromatography (TLC) using hexag-ethyl ether-acetic xylot (80:20:1) as an eluent. The analysis of the lipid composition was carried out by GC/MS on the Agilent 7000B device in the Department of Chemistry of the A.Mickiewicz University of Poznan.

Statistical processing of the obtained results was carried out by calculating the arithmetic mean and the value of the standard deviation at $0.95>P>0.80$. All determinations were carried out in 3 and 5-fold repetition. The data was processed using an IBM Pentium personal computer based on Excel application software packages.

3 RESULTS AND DISCUSSION

The main stage of our research was to determine the optimal ratio of phosphorus-containing waste in a nutrient solution for the cultivation of microalgae.

In modern biotechnology there are two directions of cultivation of microalgae:

1. Extensive cultivation in open reservoirs;
2. Intensive cultivation in closed installations under fully controlled conditions.

In industrial cultivation, conventional mineral or special media are used, in which the consumption of the main components in the process of microalgae biomass growth is taken into account.

Isolated pure cultures of green microalgae *Chlorella vulgaris* ASLI-1, *Chlorella vulgaris* ASLI-2, *Oocystis borgei* ATP from algobacterial communities of reservoirs of Turkestan region can be used for the production of algae biomass as the basis

of biological fertilizers. When selecting the optimal composition of biofertilizer with these strains and phosphorus-containing waste, it will be possible to solve the problem of replacing synthetic fertilizers with organic ones. In the production of fertilizers based on green microalgae, the selection of a nutrient medium and the process of their cultivation is of paramount importance.

Environmental degradation according to the assessment of environmental monitoring of industrial regions are turning into focal zones of major changes in the lithosphere and biosphere. In the studies of Kazakhstani scientists, it was noted that in the five-kilometer zone of influence of enterprises producing phosphorus and phosphorus fertilizers, the concentration of fluorine sometimes reaches 100-200 mg/m^3. It is also noted that under the influence of these emissions, photosynthesis of local plants decreases, vegetation suppression is observed, etc. According to the qualitative composition and harmfulness of emissions, phosphorus production enterprises belong to industrial productions that have emissions of gases or aspiration air into the atmosphere containing carcinogenic and toxic substances.

The main idea of the study at this stage was the use of solid and liquid waste from the production of phosphorus waste as components of a nutrient medium for the cultivation of microalgae. In order for the algae fertilizer to reach its full potential, the cost of growing them should be inexpensive, allowing for the production of economical mass raw materials. In this regard, a cheap source for algae cultivation is industrial or municipal wastewater, various liquid waste rich in nutrients. On the one hand, the problem of their purification is being solved, since polluted waters, either being discharged into open reservoirs or penetrating into underground and groundwater, contribute to their further pollution. Due to the well-known fact that algae successfully grow on wastewater, a possible synergistic solution is the placement and integration of the production of fertilizer with the treatment of nutrient-rich wastewater.

The processing of phosphorite raw materials into elemental phosphorus in many cases produces a large number of by-products and wastes such as phosphate slag, phosphorus sludge, etc. These wastes are formed due to the heterogeneity of the feedstock and not upgraded equipment for the preparation of raw materials for electrothermal phosphorus sublimation. For example, the processing of phosphorite raw materials for yellow phosphorus at the output forms 1 ton of phosphorus 25 - 27 kg of its compounds, 10-12 tons of slag and up to 170 kg of phosphorus sludge.

As a result of chemical analysis, the composition of phosphorus-containing slags consisting of pseudowollastonite (a-CaO×SiO $_2$), cuspidine (3CaO×CaF $_2$×2SiO $_2$), ferrophosphore Fe $_2$ P, melilite - Ca $_2$ (Al, MgSi)Si_2O_7, akermanite-Ca $_2$ MgSi $_2O_7$, rankinite 3CaO×2SiO $_2$, fluorapatite –Ca $_5$(PO $_4$)$_3$ F, whitlockite-NaF, fluorite CaF $_2$, silicocarnotite $5CaO \times P_2O_5 \times SiO_2$ was clarified.

The results of the REM analysis showed that the composition of phosphorus-containing slags contains a significant amount of biogenic elements (Figure 3), including phosphorus, whose content ranges from 0.5-21.96; potassium- 0.02- 0.46 wt.%. The presence of a number of metals necessary for phototrophic organisms as sources of growth factors and micronutrient nutrition, such as magnesium, manganese, fluorine, iron, etc. and the volume of stored waste occupying more than 16 hectares of land indicates the possibility of using these wastes as sources for industrial cultivation of phototrophs promising for biotechnological purposes.

Eleme	Cal/Mag, %
F	4.59
Na	0.37
Mg	1.54
Al	0.77
Si	10.67
P	1.42
S	0.53
K	0.46
Ca	25.37
Mn	0.06
Fe	0.25

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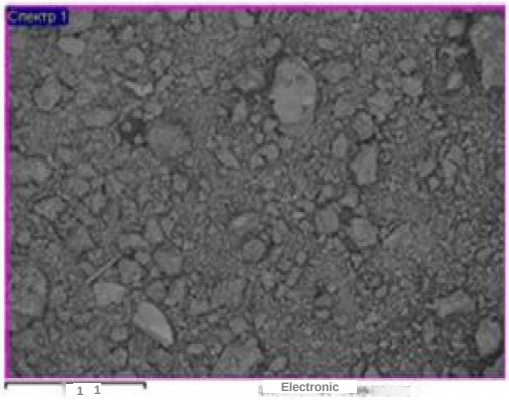

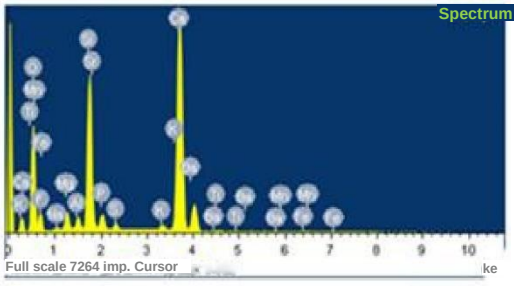

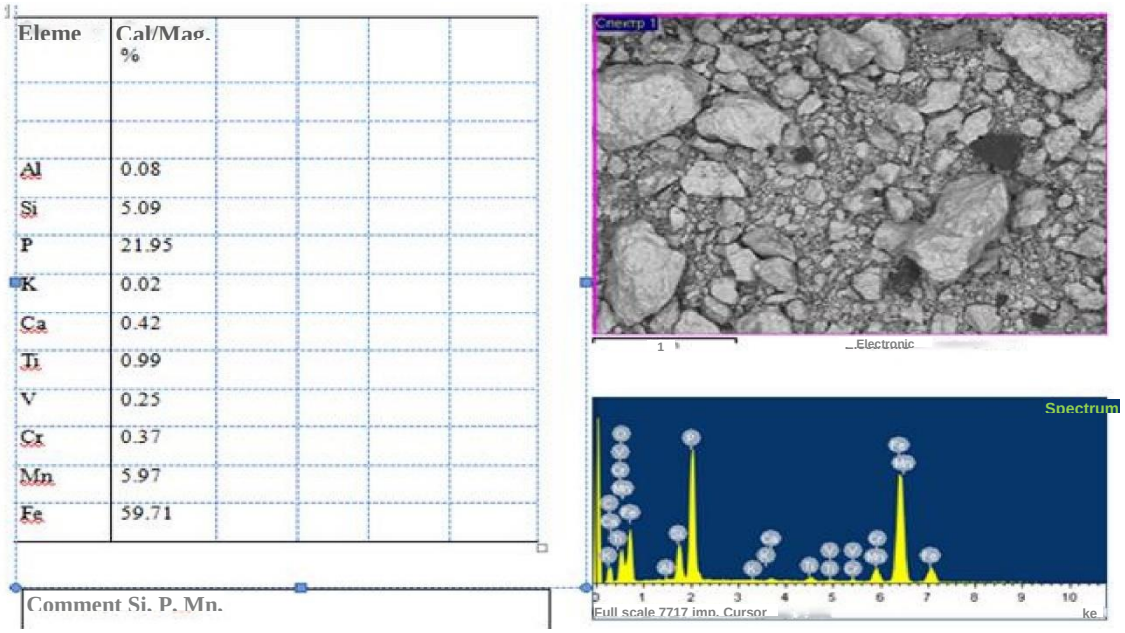

B

Figure 3: Results of REM analysis of various samples of phosphorus-containing slag.

To determine the level of moisture content of sludge at their storage sites, special depressions have been prepared to measure the water level. In spring and summer, at elevated temperatures and insolation, massive biological fouling is observed in these depressions filled with water, represented by filamentous algae *Spirogyra elongata*, *Ulothrix zonata*, in the thickness of which O. *borgei, C. vulgaris, Phacus sp. (*presumably *R.caudatus),* 3 species of *Euglena live.* The color of the water in such depressions acquires a bright green color with a sharp chemical odor, the pH ranges from 5.0-5.5.

Primary algological examination of liquid phosphorus-containing samples revealed the presence of such taxonomic groups of algae as: *Amoeba limax, Navicula sp., Meridion circulare, Oocystis sp., Diatoma sp., Anabaena sp., Chlamydomonas sp. Chlorella vulgaris*.

In the chemical composition of phosphorus-containing waste, an imbalance in the ratio N:P:K 19:2989:325 can be noted (at normal, mg/l: 220:50:200) with a clear deficiency of nitrogen and potassium group ions. As is known, *Chlorella vulgaris* and *Oocystis* cultures can use various types of salts $(NH_4)_2 CO_3$, $(NH_4)_2 SO_4$, $NH_4 NO_3$ and KNO_3, i.e. ammonium nitrogen or nitrate nitrogen, as a nitrogen source. With intensive cultivation of microalgae strains, it is necessary to maintain a nitrogen concentration in the range of 50-250 mg/l.

For a full-fledged bioconversion of phosphorus-containing waste by green microalgae, an experiment was conducted to find the optimal balance of N:P:K in the nutrient medium. Based on a comparative analysis of similar studies, it was found that the blue-green alga *Anabaena sp.* can fix free nitrogen up to 300 kg/ha. Based on this, it became necessary to include algae capable of nitrogen fixation in the algoconsorcium. Cyanobacteria *Anabaena sp.* fixes free nitrogen and further synthesizes ammonium ions, which are necessary to optimize the cultivation of *C. vulgaris* ASLI-1, *C. vulgaris* ASLI-2, *O. borgei* ATP strains in wastewater with a high phosphorus content (Table 1).

It was found that the addition of cyanobacteria to the culture of microalgae increases the nitrogen content in the finished biofertilizer by 85-87% of the initial composition using only green microalgae and, as a consequence, the density of microalgae biomass.

Table 1: Optimization of the mineral composition of biofertilizer.

Strains of green microalgae	Biomass density, CFU/ml	
	Phosphorus-containing wastewater	Phosphorus-containing wastewater+ *Anabaena sp.*
Chlorella vulgaris ASLI-1	$8.7x10^6$	$9.3 x10^8$

Chlorella vulgaris ASLI-2	7.9x10 ^{6}	9.1 x10 ^{8}
Oocystis ATP	8.2x10 ^{6}	8.9 x10 ^{8}

A mixed culture of wild algae species can be grown quite successfully on wastewater nutrients and potentially scaled up to commercial production. Algae have already demonstrated the ability to colonize wastewater with a low nutrient content in small rivers of the Turkestan region and wastewater rich in biogenic phosphorus at Kainar LLP. However, when growing these types of algae in model indoor conditions, they are limited in illumination, because artificial lighting greatly inhibits their natural processes, which is confirmed by research.

During the work, microalgae were cultivated on various percentage solutions of phosphorus-containing waste. The following cultivation mode was chosen: daylight hours 12 hours, water temperature 23 °C, pH 5.5, bubbling CO_2 for 2 hours a day. It was found that when cultured in solutions containing waste from 1.0± 0.1% to 5.0± 0.3%, the data on the growth of algae biomass does not differ from the control (Pratt nutrient medium without waste addition). An increase in the waste content above 7.0 ± 0.5% leads to the fact that the growth of the consortium of microalgae *Chlorella vulgaris* ASLI-1, *Chlorella vulgaris* ASLI-2, *Oocystis* ATP slows down, and at 15.0 ± 1.5% solution of phosphorus-containing slags and slimes, the death of algae cells is observed.

After cultivation for a week , the density of microalgae cells in nutrient media was as follows:

-1% solution – $(7.0\pm0.5)\times10^{8}$ g/cm ^{3};
- 5% solution – $(7.1\pm0.6)\times10^{9}$ g/cm ^{3};
- 10% solution – $(6.7\pm0.5)\times10^{9}$ g/cm ^{3};
- 15% solution – $(4.2\pm0.4)\times10^{3}$ g/cm^{3};
- 20% solution – $(2.3\pm0.2)\times10^{2}$ g/cm^{3}.

On the other hand, the research results of a number of scientists show different data on the productivity of algae grown on wastewater. A relatively high uptake of nitrogen and phosphorus by algae is usually described, but the total uptake of nitrogen is lower, since organic nitrogen was most likely not absorbed by algae. At the same time, the average yield obtained over the entire cultivation period was 3.3 ± 1.5 g of dry matter for 2 days. Other studies conducted with *Chlorella vulgaris* grown on wastewater report a rate of algae production of 3 g of dry matter per day per m^2, which confirms previous data. Studies report a production rate of 13 g of algae dry matter per day per m^2, when growing them on excess active sludge. At the same time, up to 94% of ammonia, 89% of total nitrogen and 81% of nitrate nitrogen were removed. However, in the following study by Kraggs, it is argued that in open reservoirs, a large variability in obtaining the amount of biomass and removing biogenic components is achievable, depending on the range of factors affecting this process: the composition of wastewater, the level of insolation, the nature of the terrain and relief, etc.

The main problem of biotechnological production of organic fertilizers is to increase the economic efficiency of production, which requires process optimization, the use of new cost-effective raw materials, improving the quality and yield of microalgae biomass.

Optimization of nutrients for the cultivation of microalgae is necessary, since the bulk of microalgae in nature has adapted to a variety of environmental conditions, such as increased concentrations of phosphorus, nitrogen and carbohydrates. Such microalgae strains need an increased concentration of these elements and in industrial cultivation. Thus, the selection of nutrients affects the economic and environmental significance, as well as the overall energy balance of the microalgae culture. Wastewater treatment with algae creates the possibility of simultaneous production of fertilizer and removal of nutrients, which will have advantages over biological removal of biogens, for example, with the help of bacteria or hydromacrophytes. Unlike cultivation of microalgae in fresh water, in the case of wastewater, the growth rate and accumulation of biomass strongly depend on the composition of wastewater. In this case, it is important to use those types of algae that are capable of consuming biogenic elements and the absence of toxic qualities, which, in the case of studies conducted, confirms the correctness in choosing strains of microalgae *C. vulgaris* ASLI-1, *C. vulgaris* ASLI-2, *O.borgei* ATP, significantly reducing the content of phosphate phosphorus and ammonium nitrogen in wastewater with sequential accumulation of biomass. On the other hand, there are known studies describing a decrease in the amount of lipids with increased growth of microalgae, since algae grown in wastewater have a low lipid content compared to the conditions of nitrogen deficiency in the medium. This conclusion contradicts Shurin's research, which shows the possibility of increasing the lipid content without lowering the biomass yield. For example, the supply of exogenous carbon dioxide increases both the lipid content and the biomass yield of *Auxenochlorella protothecoides*. The compromise between biomass productivity and lipid content can be solved by using microalgae polycultures. In this regard, the composition of the studied microalgae

strains is fully justified by the fact that each strain is capable of synthesizing certain groups of lipids.

The results of the influence of different concentrations of phosphorus-containing waste on the density of algae biomass are shown in Table 2.

Table 2: Effect of different concentrations of phosphorus-containing slags on changes in the optical density of strains *C.vulgaris* ASLI-1, *C. vulgaris* ASLI-2, *O.borgei* ATP (λ 560nm).

Nutrient medium	*C. vulgaris* ASLI-1, g/cm ^{3}	*C.vulgaris* ASLI-2, g/cm ^{3}	*O. borgei* ATP, g/cm^3
Distilled water	5.7±0.5	5.7±0.6	3.6±0.3
Pratt nutrient medium	17.8±1.5	17.8±1.5	18.1±1.7
1% solution of phosphorus-containing waste	13.5±1.1	12.4±1.2	15.4±1.5
5% solution of phosphorus-containing waste	16.2±1.6	15.8±1.4	17.8±1.7
10% solution of phosphorus-containing waste	16.1±1.5	16.2±1.5	17.5±1.6
15% solution of phosphorus-containing waste	12.6±1.1	12.4±1.1	16.4±1.5
20% solution of phosphorus-containing waste	2.1±0.1	2.2±0.2	1.4±0.1
ITA medium	19.8±1.6	19.7±1.8	20.4±2.0

According to the results shown in the table, it can be noted that the highest density of microalgae biomass was observed in 5.0%, 10.0% solutions of phosphorus-containing waste and ITA medium. In the Pratt medium, the biomass density is higher than in phosphorus-containing solutions, which is explained by the optimized composition of the first medium.

The growth and development of microalgae culture is influenced by many factors such as pH, the presence of oxygen and carbon dioxide, light, temperature. It was found that the optimal temperature for cultivation on an industrial scale is +23+27 °C, where the strains of *C. vulgaris* ASLI-1, *C. vulgaris* ASLI-2 and *O.borgei* ATP gain the maximum number of cells 9.0x10 ^{7} CFU/ml, 8.9x10 ^{7} CFU/ml and 8.7x10 ^{7} CFU/ml, respectively.

4 CONCLUSION

Thus, the optimal medium for the cultivation of microalgae *Chlorella vulgaris* ASLI-1, *Chlorella vulgaris* ASLI-2 and *Oocystis* ATP on an industrial scale is an ITA medium with 10% phosphorus-containing slag content, with such cultivation parameters as aeration using a mixture of oxygen with 2% carbon dioxide content, a daylight length of 12 hours at a temperature of +23+27 °C.

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