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Journal of Infrastructure, Policy and Development 2024, 8(8), 4817.
https://doi.org/10.24294/jipd.v8i8.4817

Article

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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CITATION

Akmaral I, Gulzhaina A, Assel T, et al. (2024). 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. Journal of Infrastructure, Policy and Development. 8(8): 4817. https://doi.org/10.24294/jipd.v8i8.4817

ARTICLE INFO

Received: 23 February 2024
Accepted: 26 June 2024
Available online: 30 August 2024

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. In this regard, the purpose of our research is the possibility of cultivating green microalgae on phosphorus production waste. During the work, traditional and modern research methods in biology were used. As a result of the work, several problems can be solved, such as the disposal of industrial waste and the production of safe biological fertilizer.

Keywords: phosphorus-containing waste; algae fertilizer; chlorella vulgaris; oocystis borgei; cultivation

1. Introduction

The Republic of Kazakhstan is one of the major agroindustrial powers of the world, which produces vegetable grains and industrial crops, annually increasing the volume of production. But, according to the Bureau of Statistics of Kazakhstan, in 2021, wheat production decreased from 14.3 million tons in 2020 to 11.8 million tons. It should be noted that the same trend was noted for barley production, where the harvest decreased from 4.8 million tons in 2020 to 3.7 million tons in 2021 (Trade Show, 2022). Such indicators are associated with a dry period in 2021, but one cannot ignore the impact of the level of land depletion due to frequent violations of agricultural technologies and crop rotation.

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 (Babkenov et al., 2023). 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 (Kunanbayev et al., 2023; Trade Show, 2022). 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 itsrelevance. 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 (Aliyat, 2022; Santoyo et al., 2021; Verma et al., 2022). Ammonium nitrate is a widely used source of nitrogen, and phosphorite flour is an effective and safemineral additive with a long-term effect. A number of studies describe an algorithm for the isolation and selection of active strains of microorganisms – the basis of future consortia (Cruz-Magalhães et al., 2022, Stuart et al., 2022, Stummer et al., 2022). It is known that microbial consortia have advantages over individual strains of microorganisms, such as stability in a wider range of environmental conditions (Trivedi et al., 2020, Tabacchioni et al., 2021). In the modern agro-industrial complex, a wide range of biofertilizers are used, which have various properties such as nitrogen fixation, plant growth stimulation (Chen et al., 2021, Kauret al., 2022) suppression of the growth of phytopathogenic microflora (Bouchard-Rochette et al., 2022, Stummer et al., 2022, Wang et al., 2019,) and resistance to various pests (Metwally et al., 2022, Spescha et al., 2023). Each time, when considering the development and use of microbial consortia, it is necessary to take into account such factors as the cost-effectiveness of creating a consortium (Qian et al., 2020), the incompatibility of microorganisms within the consortium (Bhatia et al., 2018), competition from indigenous microflora (Ramet al., 2022) and adaptation to environmental conditions (Trivedi et al., 2021). According to the classification of Bernstein and Carlson (2012), all microbial consortia are divided into artificial, synthetic and semi-synthetic. Their composition is always known, and, depending on the purpose of use, different strains of microorganisms can be combined, which makes it possible to effectively manage the processes of their use for agricultural needs (Padmaperuma et al., 2020). In general, microbial compositions can be developed based on many strategies, taking into account the complementarity of ecological niches, weather and climatic conditions, and benefits for cultivated plants through combinations of different strains with known properties. The effectiveness of the use of microbial compositions depends on the careful selection of microbial strains used, which potentially contributes to the reliability and proven consistency of their use (Soumare et al., 2020).

An important role in the development of microbial compositions is played by the choice of substrates for cultivating microorganisms. In this regard, it is necessary to note the possibility of using various technogenic wastes containing biogenic elements. The Republic of Kazakhstan occupies one of the leading places in the world in terms of the volume of generated industrial waste. According to the Bureau of National Statistics, in 2022 alone, 888.1 million tons of various waste were generated; in total, about 32 billion tons of waste were accumulated throughout the country (EnergyProm, 2024). As a result of the rapid pace of urbanization, many wastes end up within populated areas (Issayeva et al., 2020, Salim et al., 2022). Previous studies have shown the possibility of using waste from the production of phosphorus-containing mineral fertilizers for the cultivation of phototrophic microorganisms that help increase the yield of agricultural crops such as tomatoes (Tleukeyeva et al., 2021, 2022).The development of algae fertilizers based on the cultivation of microalgae, today, is one of the alternative solutions to existing problems such as replacing mineral fertilizers while increasing crop yields and improving soil quality, reducing the extraction of fossil elements with associated environmental consequences, reducing carbon dioxide emissions due to photosynthetic processes in microalgae cultivation (Cao et al., 2023; Osorio-Reyes et al., 2023; Shahid et al., 2020; Ummalyma et al., 2021). A number of algae are known that are widely used as biofertilizers to accelerate the development of agricultural crops, suchas Acutodesmus dimorphous, Spirulina Platensis, Chlorella vulgaris, Scenedesmus dimorphous, Anabaena azolla and Nostoc sp. (Ammar et al., 2022).

The use of nutrients from industrial waste for the cultivation of microorganisms is a good option for the alternative use of waste within a closed waste-free production cycle (Chaudhary et al., 2018; Scarponi et al., 2021). In this regard, the development of algae fertilizer production systems is not only a way to obtain microalgae biomass, but also an opportunity to reduce wastewater treatment costs. Thus, when studying the effectiveness of removing nutrients from slaughterhouse wastewater using Chlorella and Scenedesmus algae, it was found that the quality of the treated water was below the established standards for discharge water, and the degree of wastewater treatment ranged from 86.74% to 99.46% depending on the toxic ingredient (Bedane et al., 2023). Approaches to the use of algae species, as well as methods of their use, are different, sometimes contradictory, which is most likely associated with specific production conditions, weather and climatic conditions and the composition of wastewater. Janpum et al. (2022) proposes to immobilize microalgae on special carriers, which will reduce energy costs. In a study by Mkpuma et al. (2024) showed that in some cases the use of biofilm is more effective than an algae suspension, which significantly increases the degree of water purification from nutrients. A number of authors have proposed the combined use of algal-bacterial compositions to reduce the content of a number of toxic ingredients (Amaro et al., 2023; Bai et al., 2023; Zhang et al., 2024).

It was found that when cultivating microalgae biomass, consisting mainly of Scenedesmus, in municipal wastewater, the content of COD decreased by 69.0%, phosphates by 81.0% and total inorganic nitrogen by 91.0%. Treatment of Ocimum basilicum L. plants with microalgae fertilizer resulted in a 27% increase in plant biomass (Álvarez-González et al., 2022). On the other hand, both this and other studies noted that the use of algae fertilizers in combination with mineral or organic fertilizers can increase the yield and quality of crops, compared with options where only algae fertilizers were used. It was found that extracts of Chlorella sp. and Anabaena sp. do not have a significant effect on the germination of wheat seeds (Zhang et al., 2023), but the content of protein, chlorophyll A and B increases by 40.0%, 29.2% and 33.5%, respectively, compared to the control, which is noticeably lower than in options with mixed use of algae fertilizer in combination with mineral or organic fertilizers. A study of the possibility of cultivating Chlorella vulgaris and Scenedesmus obliquus on residual agricultural drainage water for their further use as algal fertilizers showed that the enzymatic activity of the soil significantly improves (Alvarenga et al., 2022). An increase in the activity of dehydrogenase, β-glucosidase and acid phosphatase was noted with a simultaneous decrease in soil electrical conductivity, which indicates the ability of microalgae to avoid secondary soil salinization.

An analysis of the literature data showed that the use of microalgae to produce fertilizers in order to increase the yield of agricultural crops is one of the promising areas for the development of "green" technologies, which is enhanced by the use of industrial wastes for the cultivation of microalgae, which helps reduce the content of nutrients in them.

The purpose of this study was to isolate and select active strains of microalgae and determine the optimal conditions for their cultivation on phosphorus-containing wastes in the south of Kazakhstan.

2. Materials and methods

2.1. Objects of research

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) Phosphorus-containing sludge.

(c) Phosphorus-containing wastewater.

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

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 P_2O_5, 2%–4% K_2O. 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 1a**).

2.2. Sampling technique

Water sampling was carried out using traditional methods. Water was collected into plastic containers from the surface, middle and bottom of the river. Benthic hydrophytes were collected together with stones and placed in aquariums.

2.3. Determination of elementary composition

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.

2.4. Microscopy

Microscopic studies were 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.

2.5. Microbiologic studies

Microorganisms were isolated 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.

2.5.1. Microorganisms’ cultivation

The isolation and 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:

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

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

Ashby medium for nitrogen-fixing microorganisms of the following composition, g/l: $C_{12}H_{22}O_{11}$–2.0; K_2HPO_4–0.2; $MgSO_4 \times 7H2O$–0.2; NaCl–0.2; K_2SO_4–0.1; $FeSO_4$ –2 drops 1% solution; $CaCO_3$–0.5; agar–20.0.

Tamiya medium, g/l: KNO_3–5.0; $MgSO_4 \times 7H2O$–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: KNO_3–0.10, $MgSO_4 \times 7H_2O$–0.01, K_2HPO_4–0.01, $FeCl_3 \times 6H_2O$–0.001, agar – 12,0.

ITA medium, g/l: phosphorus–containing slags–10; KNO_3–0.10; $MgSO_4 \times 7H_2O$–0.01.

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 °C+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 °C +25 °C with constant aeration.

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.

When preparing the nutrient medium, scales of the Scout-Pro brand 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 min. 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.

2.5.2. Taxonomic analysis

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.

2.5.3. Physical analysis

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.

2.5.4. Model experiments

Experiments on the use of phosphorus-containing waste for algae growth were carried out in laboratory conditions. 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 phosphoruscontaining 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.

2.5.5. 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.

2.5.6. Analysis of qualitative composition of lipid extracts

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 in Poznan.

2.5.7. Statistical processing of results

Statistical processing of the obtained results was carried out by calculating the arithmetic mean and the value of the standard deviation at $0.95 > P > 0.80$ (Britannica, 2024). 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

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. It is also noted that under the influence of these emissions, photosynthesis of local plants decreases, vegetation suppression is observed, etc. (Behjat et al., 2022). 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 (Lyon et al., 2020).

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, on tribute to their further pollution (Lin et al., 2024). 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 (Srimongkol et al., 2022).

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. (Samreen, 2019). 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 (Salim et al., 2022).

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 (Tleukeyeva et al., 2021). 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. When using mineral fertilizers, the problem of phosphorus immobilization in the soil arises, which complicates its availability to plants. As a result, the low efficiency of modern water-soluble phosphorus fertilizers creates serious environmental problems due to the accumulation of unused phosphorus in the soil. Existing forms of increasing the efficiency of phosphorus use are insufficient to solve this problem. Reconfiguring phosphate fertilizer production products will help solve many problems related not only to soil fertility, but also to the state of the environment in general (Bindraban et al., 2020). 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.

It was previously established that the composition of phosphorus-containing slags consisting of pseudovolastonitis (a-$CaO \times SiO_2$), cuspidine ($3CaO \times CaF_2 \times 2SiO_2$), ferrophosphore Fe_2P, melilite–$Ca_2(Al, MgSi)Si_2O_7$, akermanite–$Ca_2MgSi_2O_7$, rankinite $3CaO \times 2SiO_2$, fluorapatite–$Ca_5(PO_4)_3F$, whitlockite–NaF, fluorite CaF_2, silicocarnotite $5CaO \times P_2O_5 \times SiO_2$ was clarified (Issayeva et al., 2020).

The results of the REM analysis showed that the composition of phosphorus-containing slags contains a significant amount of biogenic elements, 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.

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 $(NH_4)_2CO_3$, $(NH_4)_2SO_4$, NH_4NO_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 bluegreen 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 algocomposition. 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.

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.

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: daylighthours 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 phosphoruscontaining 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:

1.0% solution – (7.0 ± 0.5) × 10^8 g/cm^3;

5.0% solution – (7.1 ± 0.6) × 10^9 g/cm^3;

10.0% solution – (6.7 ± 0.5) × 10^9 g/cm^3;

15.0% solution – (4.2 ± 0.4) × 10^3 g/cm^3;

20.0% solution – (2.3 ± 0.2) × 10^2 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 ± 0.3 g of dry matter for 2 days. Other studies conducted with *Chlorella vulgaris* grown on wastewater report a rate of algae production of 3.2 g/day per m^2, which confirms previous data. Studies report a production rate of 13.0 ± 0.1 g/day for m^2, when growing them on excess active sludge. At the same time, up to 94.2% ± 5.5% of ammonia, 89.1% ± 6.2% of total nitrogen and 81.1% ± 5.5% 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 hydro macrophytes. 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**,** 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 1**.

Table 1. Effect of different concentrations of phosphorus containing slags on changes in the optical density (λ 560 nm) of strains *C.vulgaris* ASLI-1, *C. vulgaris* ASLI-2, *O.borgei* ATP, g/cm^3.

Nutrient medium	C. vulgaris ASLI-1,	C.vulgaris ASLI-2,	O. borgei ATP,
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 9.0 × 10^7 CFU/mL, 8.9 × 10^7 CFU/mL and 8.7 × 10^7 CFU/mL, respectively.

4. Conclusion

Thus, as a result of studying strains of green microalgae for the ability to grow on phosphorus-containing waste, it was found that *Chlorella vulgaris* ASLI-1, *Chlorella vulgaris* ASLI-2 and *Oocystis* ATP on an industrial scale is an ITA medium with 10% phosphoruscontaining 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. The results of the study showed the reliability of research in this area. This method of cultivating green microalgae can be used in the disposal of industrial waste, in agricultural land and in the production of biological fertilizer.

Author contributions: Conceptualization, TA and IA; methodology, IA and AG; software, TA; validation, AA, TZ, OZ; formal analysis, TA and IA; investigation, OZ; resources, TZ; data curation, AG; writing—original draft preparation, IA and TA; writing—review and editing, TA; visualization, IA; supervision, IA; project administration, TA. All authors have read and agreed to the published version of the manuscript.

Funding: The study was carried out within the framework of the grant of the Ministry of Science and High Education of the Republic of Kazakhstan AP14869410 "Technology for the production of organic fertilizers based on the utilization of phosphorus-containing and carbon-containing waste to increase the yield of vegetable crops in the Turkestan region" (2022–2024).

Conflict of interest: The authors declare no conflict of interest.

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