

## Microalgae as a Renewable Raw Material for Agriculture, Biofuel Production and Purification of Geothermal Water from Phenols

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### ABSTRACT

The paper considers the possibility of commercial cultivation of microalgae in the republic of Dagestan (Russia) for producing the biofuel and other biotechnological goods with high value added. Microalgae can be used not only for biofuel, but also in the food and pharmaceutical industries, in agriculture as feed for farm animals and poultry, as well as for the purification of geothermal water from phenols before discharge into the sewage system. Comparative evaluation of microalgae productivity in comparison with traditional oilseeds is given. For Dagestan, located on the coast of the Caspian Sea, with mild climate and an abundance of solar and geothermal energy, the development of this technology is a very promising challenge. These conditions are the advantage for the creation of large-scale microalgae production. Biotechnology in Dagestan can become both profitable and high-tech, and innovative industry.

### 1. INTRODUCTION

Nowadays biomass of *Spirulina* is commercially produced and consumed in more than 60 countries all over the world. The largest biotech firms are located in the USA, Mexico, Thailand, India, China, Japan, Canada, and Australia, where the production of algae exceeds 1,000 tons per year. Over the past 15 years, there are 6-fold increase in *Spirulina* production. According to data for 2005, Russia in the production of *Spirulina* ranked 65th in the world.

In the former USSR, such famous companies as OOO (LLC) Agro-Victoria in the Krasnodar Krai (Director Viktoria Khmelevskaya), OOO (LLC) Gidrofit in Transnistria (Director Valeriy Prodius) and OOO (LLC) Absheron Biotechnology in Azerbaijan (Director Rovshan Makhmud) are engaged in the production of *Spirulina*. In the A.O. Kovalevskiy Institute of Biology of Southern Seas (Crimea) a pilot-industrial vortex aqua reactor is established, which has no analogues in the world. It is intended to be used in *Spirulina* production during long-term space flights, as a food and as an oxygen source, since *Spirulina* absorbs carbon dioxide and emits oxygen.

In Russia, the priority in this area belongs to the scientists of the M.V. Lomonosov Moscow State University (MGU). At present, the only enterprise in Russia growing *Spirulina* in industrial amounts is NPO (Scientific and Industrial Association) "Biosolyar MGU" (General Director Prof. Mikhail Lyamin). Industrial biotechnology of *Spirulina* cultivation was first developed at MGU in 1990 and then introduced at subsidiaries in Moldova (1992), Ukraine (1994), and Baltic (1995). Since 2010, ZAO Institut farmatsevticheskikh tekhnologiy (Institute of Pharmaceutical Technologies, Moscow), Director Doctor of Technical Sciences, prof. Kedik S.A., produces bioactive supplements based on *Spirulina* powder - Farmaspirulina.

In 2015, Rosnano together with the American company Solix Algreidents has opened R & D center Solix BioSystems Vostok on the ground of the Gubkin Russian Oil and Gas State University, where it is planned to establish the production of bioactive substances, such as SolAsta™ containing astaxanthin - one of the most powerful natural antioxidants - and Omega-3 polyunsaturated fatty acids, and also SoyLent - a product of powder nutrition, containing oil obtained from microalgae. Photobioreactor Lumian AGS260 allows to control the basic parameters of microalgae cultivation as a source of antioxidants, pigments and antibiotics. The final product is used in cosmetics, baby food, and food additives. An international team of researchers is working on the project.

The production of biomass of microalgae is the synthesis of proteins, carbohydrates, lipids, vitamins and other valuable compounds from carbon dioxide, soda, water and mineral salts in a nutrient medium, using light energy. The idea of commercial cultivation of microalgae originated in Germany in the middle of the last century, where there were attempts to get edible oils from *Diatoms*.

Microalgae growing has several advantages over conventional oilseeds cultivation. Microalgae have a short growth cycle and are unpretentious: only water, sunlight and simple nutrients are needed for their cultivation; they do not occupy fertile lands; there are no seasonal restrictions.

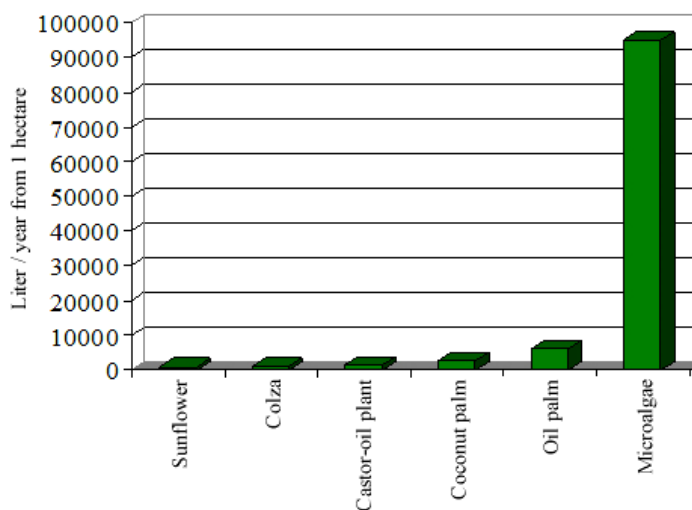
Just now, installations of open type for microalgae growing are used in Italy, Israel, Bulgaria, Mexico, Chile, Brazil, Thailand, India, China, USA (California), Central Asia, Kazakhstan, Azerbaijan, Moldova, etc. Microalgae of commercial value are the follow: *Spirulina* sp., *Chlorella* sp., *Dunaliella* sp., *Nannochloropsis salina*, *Porphyridium* sp., *Odontella* sp., *Phaeodactylum* sp., *Nostoc*, *Anabaena* (Pulz and Gross, 2004 and Kim, 2015). They are microscopic organisms cultivated predominantly in an aqueous solution of inorganic salts.

The aim of this work is to study the possibility of developing microalgae biotechnologies in the Republic of Dagestan (Southern Russia) for biofuels and valuable biologically active compounds obtaining, which can become a high-tech, profitable, and bio-oriented innovation industry for the region (Abdulagatov, 2011; Badavov, 2012; Dogeyev and Khanbabayev, 2017; <http://www.energsovet.ru/news.php?zag=1497980875>; <http://www.cleandex.ru>; [orenda.ru](http://www.cleandex.ru); [infobio.ru](http://www.cleandex.ru); [http://www.cleandex.ru/news/2017/07/27/v\\_ssha\\_otkrylsya\\_pervyi\\_v\\_mire\\_polnostyu\\_ekologichnyi\\_zavod\\_biopliiva\\_teknoblog.ru](http://www.cleandex.ru/news/2017/07/27/v_ssha_otkrylsya_pervyi_v_mire_polnostyu_ekologichnyi_zavod_biopliiva_teknoblog.ru)).

This paper is, to some extent, the continuation and development of works performed earlier in the Dagestan State Pedagogical Institute by Associate Professor N. Tumalayev. At the same time it is the beginning of a new cycle of studies on the adaptation of renewable energy technologies to large-scale production of microalgae for agriculture, biofuels production, and geothermal water treatment.

The widespread use of fossil fuel in the up-to-date industrial world resulted in problems of resource shortage and environmental pollution; and production of inexpensive biofuel is becoming one of the main tasks for mankind. Today, more than 17 countries in the world are engaged in research & development of technologies for the biofuel industry. Among them there are such world giants and corporations as Chevron, Shell, Mitsubishi, De Beers, Nestle, Boing, Chrysler Next Diesel.

Research in this area is focused on solving the main problem of renewable biofuels production: search for new strains of microalgae, which can grow rapidly and accumulate significant amounts of lipids (Horn at all, 2000). Fig. 1 presents data on the productivity of various types of plant raw materials for biofuels.



**Figure 1. Oil production from vegetable raw materials (calculation for microalgae is given)**

In the USA algal research institutes and companies are available in almost all states of the country. There are more than 500 algal enterprises, and annual investments in this industry reach \$ 500 million.

Currently, the company of Heliae from Gilbert (Arizona) has become the leader in the algae production. In April 2013 it announced the launch of the innovative technology platform Volaris for the cultivation of high-purity microalgae, creation of which took more than ten years. A new technology for obtaining aviation fuel from microalgae have already developed in the USA. Development and production are managed by two private companies - SAIC and General Atomic. The technologies allow to obtain up to 9 thousand liters of fuel from 1 hectare.

More than 38 countries have adopted programs for biofuels production at the State level. In 2012, investments in biofuels research amounted to about \$ 1.7 billion, more than 2/3 of which (about \$ 1.1 billion) were from public budgets, while private sector investments were about 500 million dollars.

The total production of biofuels including bioethanol and biodiesel, made up to 130 billion liters (4 times more than in 2003) in 2016. Three largest world markets of biofuels - the United States, Brazil and the European Union – were concentrated 85% of the world

production in 2010 with share of the USA - 48%. For biofuels, more than 100 new kinds of plant were processed in 2012 only.

The countries of Africa and South America are the most promising for expansion of biofuels production. Rapid growth of biofuels manufacture began in Asia. Currently, China is on the third place on bioethanol, and this production is expected to will be grow by more than 4% annually over the next ten years.

China is the fourth in the world country after the United States, France, and Finland which independently managed to create biofuel for aviation. So, Sinopec, the largest oil refining company in China, has already announced the first successful 85-minute test flight on the biofuels created (Katre, 2012; Knothe, 2008).

## 2. RESULTS AND DISCUSSION

### 2.1. Technologies of the Supercritical Fluid Extraction for Microalgae

The main task when manufacturing the biofuels from microalgae is to find a suitable strain with the optimal lipid composition. It is known that the lipid composition of microalgae can depend both on species, and on the condition of growing (Olmstead, 2013).

The quality of biofuel obtained is a direct result of a component composition of microalgae. Compounds with the number of carbon atoms (C16-C18) are considered to be optimal for fuel manufacturing. The associated compounds make the quality of the product worse. To obtain high-quality biofuels, selective extraction of essential fatty acids is necessary (Saraf, 2007).

The most common solvent for lipids are hexane, chloroform, a mixture of butanol and methanol, etc. However, these solvents do not suit for selective extraction. The extracts obtained by these methods require additional fractionation, which is a complicated technological problem, or purification of biofuels produced becomes necessary.

Optimum for the production of fatty acids for subsequent transesterification into biofuel is a supercritical fluid extraction, since this technique allows to influence the composition of extracts obtained by simple changes in pressure and temperature of the process (Knothe, 2005). In the Republic of Dagestan, this technology is actively developing including for microalgae. The investigations of Dagestan researchers showed all the advantages of the supercritical fluid technology in comparison with traditional ones (Aliev et al., 2013; Aliev and Stepanov, 2004, 2006; Aliev and Abdulagatov, 2017). As objects of studies, frozen microalgae *Nannochloropsis salina* had been chosen kindly provided by the Company Solix Algradients (Fig. 2).



**Figure 2. Frozen microalgae (*Nannochloropsis salina*)**

To reduce the cost of cultivated microalgae, low-enthalpy and used geothermal water containing carbonate, chloride, sulfate, phosphate salts, and trace elements are applicable as a nutrient medium. High saturation of them with carbon dioxide is a key competitive advantage decreasing production expenses.

In this case, the thermostating mode can be provided with both geothermal water and solar energy. There are many places in Dagestan with a favorable combination of such factors. Especially it should be emphasized that plantations for microalgae growing can be placed on saline and desert soils unsuitable for farming. Saline soils in Dagestan are not inferior to those used in the countries specializing in the industrial production of microalgae (USA, Israel, Japan, China).

The climatic conditions of the Caspian coast are ideally suited for mass commercial cultivation of microalgae in open ponds and obtaining both biofuel and clean food including bioactive supplements.

The main directions of the biotechnological industry of microalgae include such areas of economy as:

1. Commercial production of biofuels.
2. Manufacture of food for humans and feed supply to agriculture.
3. Bio-raw material for the pharmaceutical industry.
4. Solving environmental problems.

Significant area of microalgae application is the food industry. Extracts from microalgae are used as components of humans' functional nutrition, since in addition to nutritional properties they also have a beneficial effect on the body's functions improving health and preventing diseases. They contain a large number of physiologically necessary for humans polyunsaturated fatty acids Omega-3 and Omega-6 (Santoyo, 2006; Olmos, 2014; Schörken, 2009; Kim, 2015).

The immune-modulating effect of microalgae extracts has also been revealed. They activate the innate immune system by increasing the production of human interferon (Hirahashi, 2002). *Spirulina* extracts are active against herpes, influenza and cytomegalovirus and are able to inhibit carcinogenesis (Capelli, 2010).

The use of microalgae for feeding animals improves not only their immunity, but also the nutritional value of farm products (Caroprese, 2012). The effect of feed additives from microalgae on the health of animals has already been thoroughly investigated (Ganesan, 2011; Kannan, 2007).

## 2.2. Use of Geothermal Water for Cultivation of Microalgae for Fodder and Food Protein

Scientific and technological progress, revolutionizing industrial production, increasingly covers agriculture. Due to the lack of protein in the ration, there is an excessive feed consumption. This process has been especially noticed for the last decades. Crop production and livestock breeding are transferred to an industrial basis, and now we are talking about the technology of grain, meat and milk manufacture. A powerful fodder base is needed, which is capable to provide a sharp rise in cattle breeding.

In Russia, great importance is attached to the development of scientific foundations for the practical use of microscopic algae - *Chlorella*, *Spirulina*, etc. Interest in microalgae has increased in connection with various aspects of their practical application: for obtaining high protein fodder concentrates, for creating biological life support systems in spacecrafts, for cleaning the environment, etc. (Vladimirova, 1962; Vinberg, 1964; Gayevskaya, 1959; Gytsyk, 1963; Makhmadbekov, 1965; Muzafarov, 1968; Pinevich, 1965).

The dry matter of *Chlorella* consists of proteins - 50%, fats - 20-30%, and carbohydrates - 10-20%. In the composition of the *Chlorella* protein there are such irreplaceable amino acids as tryptophan, valine, threonine, leucine. Algae are a valuable raw material for the production of organic substances: amino acids, enzymes, hormones, vitamins, growth substances, antibiotics and other biologically active compounds for the needs of the food, chemical and pharmaceutical industries. Besides, the use of algae for wastewater treatment, air regeneration and soil fertility is of great interest.

The biomass of *Chlorella* is especially valuable due to large number of vitamins: carotene, thiamine, riboflavin, biotin, ascorbic acid, pantothenic acid, and folic acid.

The increased nutrient content makes it possible to use unicellular algae in feed and food rations. Positive results were obtained with the addition of algae to the ration of cattle and pigs (Tamiya, Malek, Bauer). Experiments on dairy cows feeding with *Chlorella* carried out in Poland have shown the possibility of replacing up to 50% of concentrated fodder with a thickened algae suspension.

Biomass of *Chlorella* is widely used in poultry feeding with a positive effect on the quality of broiler meat. Algae are also grown as feed for zooplankton, which is an important link in aquaculture.

Compared to higher plants, microalgae are high-yielding: for 7 months of cultivation their productivity in open ponds is more than 30-50 tons of dry mass per 1 hectare of water surface with a layer of water of 10-15 cm.

The search for ways to intensify the process of algae cultivation resulted in the creation of a number of original installations both in the open air, and laboratory or semi-industrial types, and high yields of biomass of unicellular algae were obtained (Mel'nikov, 2005). Experiments on the microalgae growing in commercial and semi-industrial units of an open circulating type were carried out in the Leningrad region (Pinevich, 1965), in Tajikistan (Makhmadbekov, 1965), in Uzbekistan (Muzafarov and Taubaev, 1965).

Many researchers show that the productivity of microalgae highly depends on biological and physicochemical parameters of cultivation, such as the biological specificity of the strain, the conditions of mineral and carbon dioxide nutrition, illumination and temperature.

In contrast to higher plants, algae easily adapt to different concentrations of salts. The main nutrient media is known to be proposed for the cultivation of algae include the salts:  $\text{KNO}_3$ ,  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $(\text{NH}_4)_2 \cdot \text{SO}_4$ ,  $\text{Ca} (\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ,  $\text{NaHCO}_3$ ,  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{KCl}$ . In addition, the following micro components are important in the vital activity of algae: iron, copper, manganese, cobalt, zinc, vanadium, silicon, boron, molybdenum.

The listed salts are included in the composition of artificial nutrient media, but their large-scale application is economically inexpedient. Therefore, last time much attention has been paid to the use of natural salt water as nutrient media. The first experiments on the use of mineral springs water in Bulgaria, Poland and the countries of the former USSR have yielded promising results.

Since 1970, research on the mass cultivation of microalgae in livestock and poultry farms has been carried out in Dagestan (Tumalayev and Ramazanov, 1977, 1979, Tumalayev, 1986, Tumalayev and Tumalayeva, 1987, Tumalayev, 1987, Tumalayev et al., 1987, Spruzh and Tumalayev, 1990). Positive and negative aspects of microalgae cultivation in farms have been revealed. The positive thing is that production of *Chlorella* biomass provides farms with protein-vitamin concentrate and is economically profitable. An increase in the weight of livestock and poultry, as well in the egg-laying of hens and carotene in eggs, and a decrease in mortality were noted. All costs associated with the organization of chlorella biomass production are recouped within 6 - 8 months. At the same time, there are a number of shortcomings: the absence of centralized supply of farms with all necessary salts and carbon dioxide, and a lack of qualified biotechnicians. Therefore, research was aimed at finding a cheap nutrient medium for the *Chlorella* cultivation. Accounting that algae are aquatic organisms and adapt well to various salt concentrations, nutrient media were prepared based of geothermal water.

There is a sufficient number of geothermal sources within the territory of Dagestan, which have energy, household, and balneology significance. Studies have shown that geothermal water containing sufficient quantity of biogenic elements is a favorable environment for *Chlorella* and other microalgae growing for fodder protein and vitamins.

Preliminary selection of geothermal sources was carried out according to the Institute of Geology of RAS data. Selected sources were investigated for the presence of biogenic elements, carbon dioxide, phenols, and petroleum products. The absolute majority of the investigated waters were of sulfate-chloride-hydrocarbonate-sodium and hydrocarbonate-sodium types. The geothermal water of hydrocarbonate-sulphate-sodium type with the degree of mineralization of 3.5-12 g / l, the temperature of 50-80 °C, pH 7.0-7.6, containing all the necessary macro- and microelements for cultivation of algae including carbon dioxide in the amount of 0.5-3.5 g / l are the most optimal one.

*Chlorella* was cultivated on pure geothermal water, on the Tamiya nutrient medium, on a nutrient medium prepared on the basis of geothermal water with the addition of Tamiya salts at various dilutions from 5 to 50%.

Characteristics of growth and productivity of *Chlorella* were studied under a accumulation mode of cultivation at a light intensity of 50 thousand erg. cm<sup>2</sup> and optimal temperature conditions (36 °C). The growth rate was taken into account on the quantity of cells in the population, the productivity - by the accumulation of biomass dry weight. Samples for determination of optical density, number of cells, and dry weight were taken every 5 hours during 50 hours.

Based on these data, the weight of the individual cell, the optical density, the biomass gain, and the reproduction factor of the culture were calculated.

*Chlorella* was cultivated in geothermal water using a tray type installation at a temperature of 28 °C and illumination of 15,000 lux for 16 hours a day, and stirred after every 1 hour. On the 8th day, the density of cells was 250 million / ml, and the dry weight was 5.2 g / l. The culture is characterized by intensive growth of cells in the suspension, and has a dark green color. The yield of *Chlorella* biomass in this case is twice as high as biomass obtained in known nutrient media, and on biochemical parameters it is not inferior to.

The results of *Chlorella* cultivation on various nutrient media prepared on the basis of geothermal waters are shown in Table 1.

**Table 1. Accumulation of biomass when cultivating *Chlorella* in geothermal water (grams of dry weight per 1 liter of medium)**

Variants	Geothermal sources		
	Makhachkala 160	Thernair 20	Kizlyar 4t
Pure geothermal water	1,5	1,2	1,0
5% medium	2,5	1,8	1,5
10% medium	3,2	2,6	2,2
15% medium	3,8	3,0	2,5
20% medium	5,2	3,6	2,8
25% medium	5,2	3,9	3,4
30% medium	5,0	4,2	3,8
35% medium	4,8	4,6	3,8
40% medium	4,5	4,6	4,2
45% medium	4,5	4,5	4,0
50% medium	4,0	3,8	3,6

The greatest amount of biomass was accumulated during cultivation *Chlorella* in geothermal water of Makhachkala 160 with supplying 20-25% of Tamiya salts. The high productivity is explained by the availability of easily assimilable salts, microelements, carbon dioxide and the optimal pH of the environment. The increase in the biomass of *Chlorella* cultivated in the geothermal water of Thernair 20 greatly depends on the concentration of phenols and oil products, which hinder intensive photosynthesis. Large-scale cultivation of *Chlorella* and other microalgae in geothermal water will not only provide the farms with cheap protein fodder, but also prevent contamination of environment with waste water.

Preliminary calculations have shown that the cost of biomass of *Chlorella* grown in geothermal water is two times less compared to the biomass obtained in the Tamiya medium. When *Chlorella* is cultivated on geothermal water, there is no need for imported carbon dioxide, as one of the factors of intensification of biomass accumulation.

A nutrient medium based on thermal mineral water allows to grow a cheap biomass of microalgae by saving mineral salts and carbon dioxide. In addition, the heat of geothermal waters allows to grow microalgae all the year round.

### 2.3. Purification of Geothermal Water from Phenols

Algae can be used for the disposal of organic waste. This direction is very promising, since algae consume phenols, nitrates, phosphates and reduce the number of bacteria and toxins in the water. Application of algae for sewage treatment in food enterprises; fish, livestock, and poultry farms; and slaughterhouses (Lijie Zhang et al., 2017; Olsson et al., 2014; Trifonov, 2009, 2010) have good prospects.

In experiments on the cultivation of *Chlorella*, microalgae cells are found to reduce the amount of phenols in geothermal water to the value of 0.002 mg/l. Therefore, the ability of the *Chlorella* culture to dephenolize and demineralize geothermal water must be used when discharging spent geothermal water.

When operating the Thernair wells in Makhachkala, phenols (50 mg/l) in geothermal water were found to exceed the maximum permissible concentrations. There was a problem of dephenolization of geothermal water before their discharge into the sea. It is known that various bacteria, actinomycetes, fungi and algae oxidize phenol, and its concentration in the waste water decreases. Algae and bacterial complexes had been proposed for cleaning geothermal water from phenols after primary heat removal (greenhouses, communal facilities, GeoPP) before their discharge (Yegorov, 1946; Rogovskaya and Lasareva, 1959; Labinskaya, 1960; Vinberg and Ostapeneya, 1961; Sivko, 1961; Putilina, 1964). Geothermal water of hydrocarbonate-sodium type are characterized by high mineralization (22-24 g / l). The content of microcomponents is: bromine - 52-65 mg / l, iodine - 8-13 mg / l, magnesium - 15-16 mg / l, ammonium - 22-55 mg / l, HCO<sub>3</sub> - 600 mg / l. The concentration of naphthenic acids ranges from 1.6 to 31.1 mg / l, benzene - from 0.8 to 2.4 mg / l.

A characteristic feature of the studied waters is an increased content of phenols - from 2.5 to 50 mg / l. Carbon dioxide contained is 4.6 - 6.6% (1.2 g / l), pH - 8.6. The water temperature before discharge is 35-38 °C. Dehydration of geothermal water was carried out with the help of algal and bacterial cultures: *Chlorella vulgaris*, *Ps. fluorescens*, *Symplica thermalis*. Phenol oxidizing activity of the studied cultures was determined in various nutrient media containing phenol in concentrations up to 50 mg / l. The nutrient medium of Tamiya, the mineral mixture and geothermal water were poured into flasks and inoculated with algae and bacteria cells at a rate of 1 million / 1 ml of medium; then incubated for 10 days at temperature 35-40 °C and pH 7.2-7.4, with illumination of 12-15 thousand lux.

Stirring was carried out by air flow without addition of carbon dioxide. On the 3rd, 5th, 7th and 10th day of incubation, the amount of phenol was determined, as well as algal and bacterial cells. Sterilized phenol-containing geothermal water served as control medium. There was four-fold repetition of the experiment.

When passing algal and bacterial cultures through the phenol-containing nutrient media, the cultures were selected capable to grow in media containing up to 50 mg / l of phenol. Oxidation of phenols by algal and bacterial cultures in different media occurred with varying intensity (see Table 2). The most intensive phenol oxidation was observed in the Tamiya medium, the lowest – at application of geothermal water (Tumalayev, 1986).

**Table 2. Oxidation of phenol by algal and bacterial cultures in various media**

Nutritive medium	Phenol, mg / l
1. Tamiya medium	0,005
2. Mineral water	7
3. Geothermal water	24
4. Sterilized mineral water	50

Phenol oxidation in geothermal water depends on the biochemical activity of algae and bacterial cultures, and the term of incubation. The maximum decrease in the concentration of phenol (5 mg / l) occurs on the 10th day of the combined incubation of *Chlorella vulgaris* and *Ps. Fluorescens*. The weak activity of algal and bacterial cultures with respect to phenol is associated with deficit of nitrogen and phosphorus in water. To intensify the process of biochemical oxidation of phenol, ammonium sulfate - 0.5 mg / l, and chicken manure - 2 mg / l or fecal liquid - 10 mg / l were added to geothermal water as a source of nitrogen. A sharp decrease in phenol (by 99.95%) was noted in geothermal water with fecal fluid on the 10th day of joint incubation of *Chlorella* and *Pseudomonas*. In other cases, the results were similar or slightly different from the control ones (Figure 3).

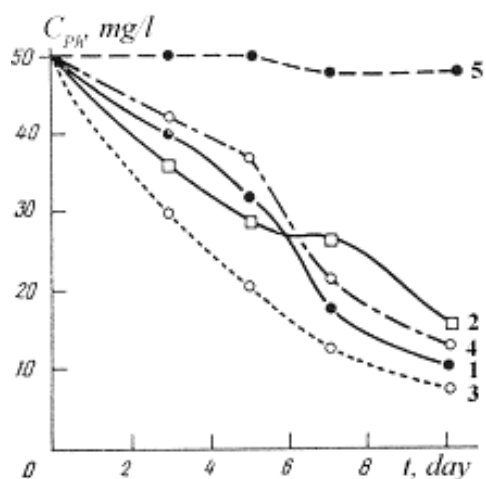


Figure 3. Dynamics of phenol oxidation in geothermal water with the addition of *Chlorella* (1), *Pseudomonas* (2), *Chlorella* and *Pseudomonas* (3), Blue-green algae (4), and in control medium (5).

The growth of algal and bacterial cells in phenol-containing geothermal water correlates with the incubation time and the phenol concentration (Fig. 4). Intensive growth in the number of algal and bacterial cells and oxidation of phenol was observed in the joint cultivation of *Chlorella* and *Pseudomonas* that is explained by their mutually stimulating effect.

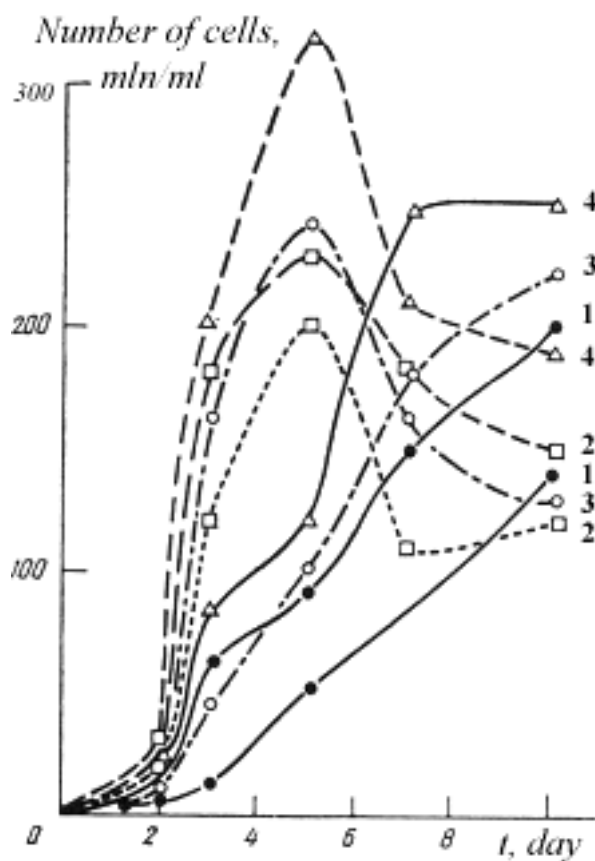
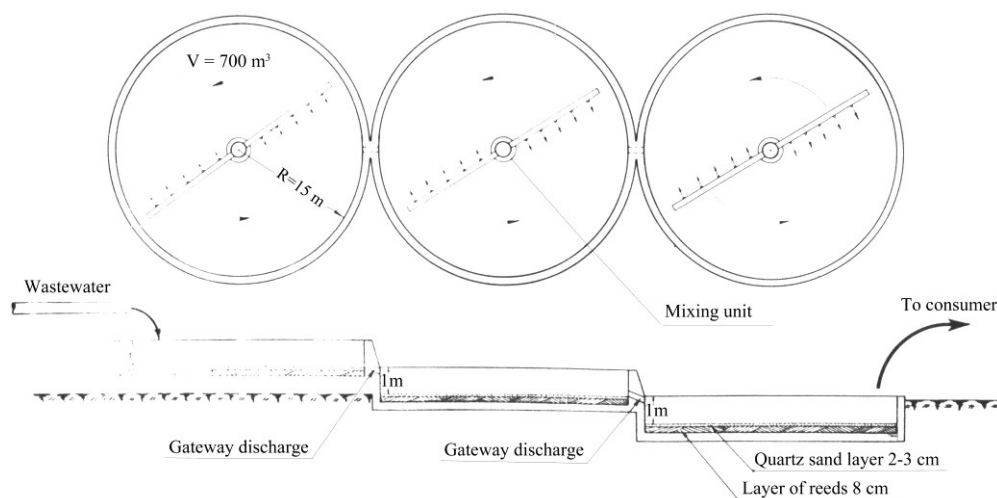


Figure 4. Growth of biomass of algal and bacterial cells in thermomineral water: the number of *Chlorella* cells in geothermal water with phenol (1), without phenol (1'); *Pseudomonas* with phenol (2), without phenol (2'); *Chlorella* in co-cultivation with *Pseudomonas* with phenol (3), without phenol (4); *Pseudomonas* with *Chlorella* and phenol (3'), without phenol (4').

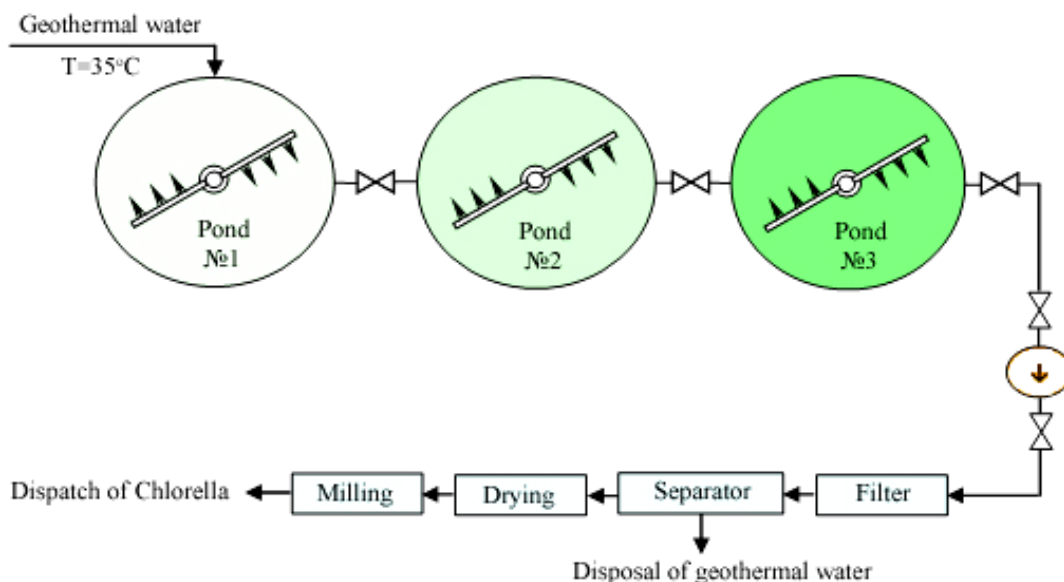
For dephenolization of geothermal water at the Makhachkala Greenhouse, a three-basin plant was proposed, in which, after the initial removal of heat, algae and bacterial cultures were grown. The installation consisted of three round concrete ponds (d - 30 m, height - 0.5

m) connected with gateways. At the bottom of the basin a layer of reeds of 3 cm high was laid, covered with a 2-3 cm layer of quartz sand (see Figure 5).



**Figure 5. Diagram of a basin plant for the cultivation of algal and bacterial cultures**

Cascade location of the ponds was used. Each subsequent pond was 30 cm lower than the previous one. The total working capacity of the installation was 2100 m<sup>3</sup>. A suspension of algal and bacterial cultures was mixed with the agitator for 10 minutes every hour. All three pools were equipped with a stationary electric motor of the AO-52-4 brand (1400 rpm). The installation was designed for 24-hour



**Figure 6. Diagram of basin plant for geothermal wastewater dephenolization.**

The first pond was filled in with geothermal water with introducing the fecal liquid, then was inoculated with a suspension of *Chlorella* and *Pseudomonas* at a rate of 20 million cells per 1 liter of water. After three-day cultivation, the content of the first basin were released into the second one, and the first pond was filled with geothermal water. The content of the second pond after the three-day incubation was released into the third pool, whence after three-day cultivation, 50 m<sup>3</sup> of the suspension was pumped out and sent to consumers or poured into the drain. In the process of biochemical exposure of algal and bacterial cultures, the concentration of phenol in geothermal water (at its initial content of 50 mg / l) at the end of the third day decreased in the first basin by 20%, in the second - by 50% and in the third - by 99.5%.

The biomass of algae and bacterial cultures is of considerable interest as a protein-vitamin concentrate for animals and poultry. The yield of dry biomass is 1.8 g / l with content of protein 45.5%, fat 29.2%, and carbohydrates 15.6%. There are carotene, thiamine, riboflavin, pyridoxine, nicotinamide, and ascorbic acid in biomass. The protein contains more than 18 amino acids. Thus, the biochemical method of thermomineral water purification from phenols ensured the non-waste use of heat and chemical elements of geothermal water, and prevented water pollution when discharging into the sea.



### 3. CONCLUSIONS

We have identified the optimal parameters for the supercritical carbon dioxide extraction of the lipid fraction from the microalgae *Nannochloropsis salina*, a promising cyanobacteria for biodiesel production. The component composition of fatty acids forming triacylglycerides of the lipid fraction was found, and the content of a large number of polyunsaturated fatty acids was established, which is promising for the medicinal use of vegetable fats obtained from these microalgae.

Due to the absorption ability of microalgae with respect to carbon dioxide, they can be used as "traps" of carbon dioxide in thermal power plants. For example, using carbon dioxide and carbon monoxide emissions generated during the year at Makhachkala CHPP (18 MW), it is possible to produce up to 25 thousand tons of microalgae and reduce the accumulation of carbon dioxide in the atmosphere of the Capital of Dagestan by 50 thousand tons.

Microalgae are undoubtedly a product with high added value, and they are valuable raw material, since their cells contain many useful substances with high biological activity.

These advantages and state significance are a good prerequisite for the establishment in Dagestan of commercial production of microalgae for biofuel and valuable matter manufacture. Biotechnology can become not only profitable, but also a high-tech and innovative branch of the economy for the Republic of Dagestan.

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