Prospects of geothermal water Use in cultivation of *Spirulina*

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**Abstract:** *Spirulina* has been studied due to its commercial importance as a source of essential amino acids, protein, vitamins, fatty acids etc. Most of the culture systems in use today are open ponds. The new approach proposed in this paper is to use the geothermal water as a medium for microalgae cultivation. Poland has beneficial conditions for wide geothermal use, as one of the environmentally friendly and sustainable renewable energy sources. In the planned research, geothermal water could be used to prepare microalgal culture medium, to heat greenhouses with bioreactors used for the growth of *Spirulina*, to dry the obtained biomass, as well as to heat the ground in foil tunnels. Using geothermal water gives the possibility to produce algae in open ponds covered with greenhouses and to cultivate plants during winter. The obtained algae can be used for the production of algal bio-products (e.g. homogenates), having the potential application in plant cultivation.

**Keywords:** microalgae, geothermal water, cultivation, bio-based fertilizer, crop plant

1 Introduction

*Spirulina*, a blue-green cyanobacterium found in alkaline water [1], has been used by human for thousands of years [2]. It was consumed by Mexican and African people [1,2] as food and nutritional supplement [3]. This microalga is cultivated and still widely used in many countries around the world as healthy food [2]. It is the richest natural source of many compounds, e.g., 60−70% protein, minerals, fatty acids (e.g., γ-linolenic acid) [4], polysaccharides [1], pigments (chlorophyll, carotenoids, phycobilins) and nearly all essential vitamins (e.g., A, B₁, B₂, B₆, B₁₂, C, E, biotin, folic acid) [5]. Environmental conditions have an influence on productivity or the chemical composition of *Spirulina* cultivated in synthetic medium [6].

The interest in microalgae is increasing because of bioactive compounds that have various therapeutic properties [1]. Preparations based on microalgae may be used in the treatment of many diseases [1,4], e.g., cancer [7], anemia [8], diabetes [9], allergy [10], antihypercholesterolemia [11], HIV [12]. It also indicates radioprotective [13] and hepatoprotective effects [14], enhances the immune system [15] and DNA repair synthesis [16].

The toxicological studies have proven that *Spirulina* is safe for human. It has been sold as pills or a health drink for more than 20 years [15]. Algae could be an alternative functional constituent of food [17].

Additional application of *Spirulina* include: animal feed [18], biofuels [19], fertilizers [20], wastewater treatment [21], removal of heavy metal ions [22], certainly could be applied in pharmaceuticals, dietary food supplements [18] and cosmetics, as well [5]. Below, Figure 1 shows algal biomass production (inputs and potential outputs) [23].

The aim of this study was to evaluate the possibility of using geothermal water to prepare microalgal culture medium and heat greenhouses with photobioreactors designed for *Spirulina* cultivation. The planned experiment will be conducted in Bańska Niżna (Poland) in collaboration with The Mineral and Energy Economy Research Institute of Polish Academy of Sciences (PAS...
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MEERI). Grown and harvested biomass will be dehydrated by means of geothermal drying. Dry microalgae will be designated to produce algal homogenates. Obtained preparations will be utilized as bio-product for crop production on selected ground area heated by geothermal energy.

2 Cultivation of Spirulina

Germany (during World War II) were the first country where the large-scale cultivation of microalgae and use of the harvested biomass for the production of useful products [2] e.g., production of lipids for energy using flue gasses, production of various biochemicals, antimicrobial substances and plans for the use in sewage treatment [24] was conducted. Commercial large-scale culturing and harvesting of Spirulina commenced in the early 1970s in Lake Texcoco, Mexico by Sosa Texcoco S.A. [18]. Microalgae cultivation is still of great interest among consumers due to Spirulina properties [25]. Currently, the production of microalgal biomass reaches 5 000 tonnes of dry matter every year, it is mostly produced in open ponds. It leads to a turnover of approximately $1.25 \times 10^9$ USD/year [5,26]. Closed bioreactors produce only a few hundred tonnes of biomass. In recent years growing interest is observed in the possibility of using biomass for low cost biofuel production [26].

The efficiency of outdoor microalgae cultures in open ponds depends on a number of factors, e.g., temperature, nutrients, $O_2$ and $CO_2$, physical factors (quality and quantity of light), biotic factors (pathogens, competition with other algae), operational factors (shear produced by mixing, depth, dilution rate and harvest frequency) [27,28], alkaline pH and high salt concentration [29]. Optimization of parameters which affect the amount of light received by the cells, such as: pond depth, cell density, turbulence and the dilution cycle allows better use of sunlight, resulting in higher productivities. The process can be carried out in large outdoor culture ponds where the modification of those parameters is possible [27]. One of the major factors that controls the growth of Spirulina is temperature. The optimum range of this factor lies between 30 and 35°C [30]. Light is a second important parameter. While the illumination levels are too low (photo-limitation) or too high (photo-inhibition) reduction of growth is observed. In between these two extremes, specific growth rate ($\mu$, day $^{-1}$) becomes independent of light, in the light saturation range [31]. The primary nutrients and micronutrients should be provided for proper growth. It can be then costly if they need to be added in great amounts [32]. In order to avoid the cost and improve the culture conditions, cultivations facilities with transparent polyethylene which prevents from heat loss and contamination should be used [30].

Microalgae cultivation can be carried out in open or closed systems (photobioreactors) [33]. The outdoor cultures allow to obtain large amounts of biomass at low costs. This system does not require control of the environmental conditions (light and temperature) [25]. Among the open systems, the natural waters (lakes, lagoons, ponds) and artificial ponds or containers can be mentioned [33]. Depending on the shape, size and type of agitation and inclination, the open pond systems can be divided into three types: sloped, raceway and circular pond [34]. Open ponds are mainly characterized by easy construction and operate similarly to photobioreactors. However, these systems show several disadvantages like: poor light utilization by the cells, diffusion of $CO_2$, evaporative losses, requirement of large areas of land and inefficient stirring mechanisms (mass transfer rates are very poor resulting in low biomass productivity). The commercial production of algae in open systems have been restricted to only those organisms that can grow under extreme conditions because of the possibility of contamination (e.g., by predators or other heterotrophs) [33].

Closed systems, commonly known as photobioreactors, can be designed in a variety of configurations. The benefits of using these systems are associated with higher volumetric productivity than open ponds, more optimal use of the cultivation area, better capture of radiant energy and variable energy consumption values for mixing and gas/liquid mass transfer [35]. Another advantages of closed systems are: facilitated temperature control, the ability to improve illuminance or when the concentration of cells is low, eliminate or reduce
contamination [28]. Most outdoor photobioreactors are
categorized by largely exposed illumination surfaces [33].

Bioreactors allow for the production of strains rich in
high value products and taking advantage of the metabolic
flexibility of microalgae. The rate of generation of the
desired product can be increased by setting the appropriate
culture conditions. The design of closed photobioreactors
must be carefully optimized for each individual algal
species, according to its unique physiological and growth
characteristics [36].

In laboratories and in industrial scale, different
cultivation systems for microalgae cultivation have been
tested [35] (Table 1).

The most common system used for cultivation of
microalgae are open ponds [37]. Currently, much attention
in bioengineering and biotechnology [37] is paid to the
development of appropriate closed systems, such as: flat,
cylindrical, vertical column and internally illuminated
photobioreactors, which are the alternative for open
ponds [33] and show promise for application in large-scale
microagal culture [37].

3 Prospects of geothermal water
use in cultivation of Spirulina

Direct-use of geothermal energy is one of the oldest, most
common and versatile form of utilization of geothermal
energy [39]. Geothermal waters were first used in
greenhouse heating in Iceland in the 1920s. Currently,
hundreds of hectares of greenhouses are operating
throughout the world [40]. In Greece, the use of geothermal
waters for greenhouse heating started in early 1980s [41]
and in the cultivation of Spirulina in the late 1990s. The
cultivation of microalgae requires CO₂, most of which come
from the dissolved CO₂ in the geothermal waters
(4 kg of pure CO₂ per cubic meter). Thermal waters exploited
in Greece cannot be directly used in the cultivation ponds
because they contain about 0.50 mg L⁻¹ As [42]. In the
cultivation of microalgae in Poland (temperate climate)
it is possible to use geothermal resources. The country is
characterized by the heat flow values from 20 to 90 m W m⁻²,
while geothermal gradients vary from 1 to 4°C/100 m.
Geothermal water and energy resources in Poland are
associated with formations of various ages in the Polish
Lowlands, the Inner Carpathians, some locations in the
Sudetes region, the Outer Carpathians and the Carpathian
Foredeep [43] (Fig. 2.) (based on [44], updated). The
operating sources have depths of 1-4 km. Quarryed
water has a temperature in the range of 30 to 130°C and
salinity from 0.1 to 200 g L⁻¹. The proven geothermal water
reserves, calculated on the basis of flow tests from one
well, range from several to 150 L s⁻¹. The best conditions
are in the Polish Lowland province and in the Podhale
region (Inner Carpathians) [45]. The chemical composition
of the groundwater is influenced by several factors,
including the lithology of the aquifer rocks, the water-rock
thermodynamic equilibrium and the circulation of ground
waters. Geothermal waters exploited in Poland have
different physico-chemical properties [46]. Geothermal
waters are appropriate for wide spectrum of direct uses
for geothermal heat pumps, space heating, greenhouse
heating, aquaculture pond heating, agricultural drying,
cooling and snow melting, bathing and swimming,
industrial uses [47]. In the last decade of the 20th
century, the use of geothermal energy for heating
purposes was initiated [48]. The most common direct use
of geothermal energy is space heating, for what moderate
and low temperature waters are best suited [49]. However,
geothermal drilling is difficult because of the nature of the
rock being penetrated, corrosive nature of the fluids and
the high temperatures [40].

Studies on the use of geothermal water in the culture
of microalgae will be made in agreement with The Mineral
and Energy Economy Research Institute of the Polish
Academy of Sciences (PAS MEERI). This Institute, in 1993,
in Bańska Niżna (southern Poland, Podhale Basin) built
and put into operation the first geothermal plant in Poland
[46,48]. At that time geothermal waters were extracted
based on a doublet (exploit Bańska IG-1 well and inject
Biały Dunajec PAN-1 well) principle. The energy from
the water first heats about 200 residential houses in the
Bańska Niżna and Biały Dunajec locations, in addition to
a school, church, timber seasoning facility, thermophilous
fish farm building, greenhouse and foil tunnels [50,51]. The
thermal waters are carried to the surface without using any
pumps and are then directed to plate heat exchangers [52].
Currently, it is worthy to note, that the Podhale geothermal
district heating system belongs to the largest in Europe
for its geothermal capacity and heat production with
a target capacity of 40 MWt and heat production of about
300 TJ yr⁻¹ [43]. Exploitation approach Bańska IG-1 has
total depth 5,261 m (the geothermal water reservoir is
2,565 m below ground level) [46]. Water outflows has the
temperature about 82–86°C [50], yield from a single well
can reach 550 m³ h⁻¹ (Bańska PGP-1 well) [53] and the
pressure is about 2.7 MPa [52]. In Podhale, geothermal
waters are extracted from carbonate formations
of the Middle Eocene and from Middle Triassic
limestones and dolomites. These exhibit relatively
low mineralisation – from 2.358 g L⁻¹ (Na-Ca-SO₄-Cl
hydrogeochemical type) in the Bańska IG-1 well to 3.150 g L⁻¹ (SO₄²⁻-Cl-Na-Ca) type in the Bańska PGP-1 well [46].

On the area of the Institute, there are located greenhouses (Fig. 3) with shelves (previously used for plant cultivation) (Fig. 4), which can be used to culture microalgae, with dimensions: length 344, width 144, depth 20 cm (2 shelves) and length 464, width 143, depth 20 cm (10 shelves). Small depth of tanks will provide optimal access to sunlight of algal cells. According to the available literature, the productivity of raceway ponds should theoretically be in the range of 50–60 g m⁻² day⁻¹, but in

| Type of cultivation system | Advantages | Disadvantages | Source |
|----------------------------|------------|---------------|--------|
| Open ponds (e.g., raceway ponds) | - appropriate illumination (10–50 cm deep) low-energy-consuming paddle wheels for gas/liquid mixing and circulation - the culture medium is directly exposed to the atmosphere - allowing liquid evaporation (regulate the temperature of the process) - made of less expensive materials - construction involves lower costs - requires less energy for mixing - better turbulent flow - shallower culture depth | - limited to the type of microalgae that can be used for cultivation - larger area required - the lower efficiency of light utilization - the poor gas/liquid mass transfer - the lack of temperature control - the high risk of culture contamination - the low final density of microalgae - significant evaporative losses - CO₂ used not efficiently - biomass productivity is lower than in closed cultivation systems - costs of harvesting algal biomass are high | [18,32,35,37] |
| Flat-plate bioreactor | - large illumination surface area - suitable for outdoor cultures - good for immobilization of algae - good light path - high productivity - relatively cheap - easy to clean up - readily tempered - low accumulation of dissolved oxygen - easily built - provision of an open gas transfer unit | - scale-up requires many compartments and support materials – difficulty in controlling culture temperature - possibility of hydrodynamic stress to some algal strains - algal wall adhesion - systems are not amenable to sterilize - incompatible with the shelf industrial fermentation equipment | [18,33,37,38] |
| Vertical-column bioreactor | - high mass transfer - good mixing with low shear stress - low energy consumption - high potential for scalability - easy to sterilize - readily tempered - good for immobilization of algae - reduced photoinhibition and photo-oxidation - simple cultivation - no moving parts - good solid suspension - homogenous shear - less land is required | - small illumination surface area - construction requires sophisticated materials - shear stress to algal cultures - decrease of illumination surface area upon scale-up - high fragility - low versatility of the material in stake | [33,37,38] |
| Tubular bioreactor | - large illumination surface area suitable for outdoor cultures (large-scale) - fairly good biomass productivities - relatively cheap - better pH and temperature control - protection against culture contamination - good mixing, less evaporative loss | - gradients of pH - dissolved oxygen and CO₂ along the tubes - fouling - some degree of biofilm growth requires large land space - overheating - high material and maintenance costs - limited tube diameter and length | [18,32,33,37] |
practice, productivities of 10–20 g m\(^{-2}\) day\(^{-1}\) are difficult to achieve [32]. In addition, during winter, this alga cannot grow in open ponds, except in the tropics. Geothermal energy could be used to heat up the greenhouse and maintain the optimum temperature for growth of *Spirulina* by heat exchangers which are located below and next to the tanks.

In Geothermal Laboratory of the PAS MEERI there is located a geothermal water desalination installation. Objective of the process is to produce drinking water. In this process a concentrate is obtained. The installation is supplied with water at a temperature of around 35°C [46]. Schematic diagram of the desalination system is shown in Fig. 5. (based on [44]).

Below the composition of the geothermal water, tap water and concentrate (essential components in the culture of *Spirulina*) is reported. According to data presented in Table 2, the geothermal water has a higher concentration of ions in comparison to tap water e.g., Ca, K, Mg, Na. These elements are important medium components. The pH value of tap water is 6.97 and for geothermal water is 7.41. The application of thermal water in cultivation of *Spirulina* can reduce the cost associated with the use of medium components (e.g., \(K_2SO_4\), NaCl or \(MgSO_4 \times 7H_2O\)).

In Table 3 the composition of medium for *Spirulina* cultivation in different water (included their elemental composition) is presented. Table 4 contain the composition of trace metal solution. The cost of cultivation of microalgae in photobioreactor with the volume 1500 L (using the pure reagents from CHEMPUR) will amount to 153.34 USD. The renunciation of using the geothermal water instead of the tap water is 7.1 USD. Use of a concentrate proved to be uneconomical. In order to calculate the composition of the medium mathematical software Solver was used.

Grown and harvested biomass will be dehydrated by means of geothermal drying. Produced algae homogenate can be utilized as microelement fertilizer, biostimulant or bioregulator for crop production on selected ground areas heated by geothermal energy (Fig. 6.). This solution allows plant production also during the winter.
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The current attention has been paid to decreasing pollution sources and to the need to adopt eco-friendly agricultural practices for sustainable agriculture [54]. The use of bio-products can improve this situation. These products can be divided into 3 groups: biostimulants, plant growth regulators and biofertilizers. Biostimulants are materials that stimulate plant development, while plant growth regulators control plant growth processes, and biofertilizers provide nutrients to plants.

Table 2: The composition of the geothermal water, tap water and concentrate obtained through desalination of water from Bańska IG-1 well.

| Element | Geothermal water | Tap water | Concentrate |
|---------|------------------|-----------|------------|
| Ca**   | 231              | 79        | 645        |
| Co**   | 0.0001           | 0.000050  | 0.0014     |
| Cu**   | 0.045            | 0.0048    | 0.019      |
| Fe**   | 0.058            | 0.015     | 28.7       |
| K**    | 76.8             | 5.1       | 145        |
| Mg**   | 46               | 10        | 123        |
| Mn**   | 0.096            | 0.0035    | 0.434      |
| Mo**   | 0                | 0.00086   | 0.2        |
| Na**   | 433              | 14        | 1794       |
| Zn**   | 0.0079           | 0.42      | 0.068      |
| Cl***  | 696              | 1.6       | 2433       |
| NO3*** | 2.2              | 4         | n.a.       |
| SO4*** | 868              | 33        | 2819       |
| HCO3***| 313              | 281       | 404        |
| CO2*** | <0.5             | <0.5      | <0.05      |
| PO4*** | 0.229            | 0.02      | 0.662      |

LLD: below the limit of detection; n.a.- not analyzed, Analyzed technique: *ICP-OES/ICP-MS, **Cary UV-vis
***Alkalinity, chloride and nitrates ion concentration were determined by titration following accredited testing procedures.

4 Algae in agricultural products

The current attention has been paid to decreasing pollution sources and to the need to adopt eco-friendly agricultural practices for sustainable agriculture [54]. The use of bio-products can improve this situation. These products can be divided into 3 groups: biostimulants, plant growth regulators and biofertilizers. Biostimulants are materials that stimulate plant development, while plant growth regulators control plant growth processes, and biofertilizers provide nutrients to plants.

Figure 3: Greenhouse.

Figure 4: Shelves that can be adapted for cultivation of microalgae.

Figure 5: Simplified diagram of the thermal water desalination process (based on [44]).
(other than fertilizers) which promote plant growth when are applied in small quantities. They are also referred to metabolic enhancers [55]. In agriculture are used to improve root growth and increase the crop yield [56].

Algae contain growth regulators (auxins, cytokinins and gibberellins) which influence division cycle, cell growth, expansion, nutrition and maturity. Compounds found in algae induce the wide range of growth responses. It implies the presence of more than one group of plant growth-promoting substances/hormones [55]. Bio-based fertilizers deliver to plants nutrients of biological origin and activate the soil fertility to produce higher crop yield [57]. Crops production of vegetables needs essential nutrients from the soil especially N and K [58]. This bio-product adds nutrients through the natural processes of phosphorus solubilization and nitrogen fixation. Additionally stimulates plant growth through the synthesis of growth promoting substances. Bio-based fertilizers could be used instead of chemical fertilizers and pesticides, or as their supplementation, what brings economic and ecological benefits (soil health and fertility) [59].

Currently, commercially used biopreparates contain mostly macroalgae [60] e.g., Kelpak, Wuxal and Ascofol [56]. They are used as growth stimulants in agricultural crops and as amendments in crop production systems [60]. According to our knowledge, *Spirulina* has not commercially been used as an organic fertilizer yet. In the literature, there are several reports that show the beneficial effect of these microalgae on the growth and development of plants. Studies on the effects of *Spirulina* on the cultivation of pepper (*Piper L.*) showed a favorable effect [58]. Authors used dry biomass for preparation of the foliar fertilizer at the rate of 80 g L⁻¹. In the test group, first yield was higher when compared to those fertilized with compost and NPK fertilizers. This result could be due to high content of free amino acids, macro- and microelements in the *Spirulina* product which are absorbed faster than the nutrients from the soil by using mineral fertilization or compost. Foliar compost application showed that *Spirulina* solution

![Figure 6: Geothermal heating foils.](image)

| Component | Quantity used (g) |
|-----------|------------------|
|           | Distilled water | Geothermal water | Tap water |
| NaHCO₃    | 13.61            | 13.18            | 13.22     |
| Na₂CO₃    | 4.03             | 4.029            | 4.029     |
| K₂HPO₄    | 0.50             | 0.50             | 0.499     |
| Solution I (500 mL) |
| NaNO₃     | 2.5              | 2.49             | 2.495     |
| K₂SO₄     | 1.0              | 0.657            | 0.977     |
| NaCl      | 1.0              | 0.208            | 1.236     |
| MgSO₄ × 7H₂O | 0.2            | 0               | 0.1475    |
| CaCl₂ × 2H₂O | 0.04           | 0               | 0         |
| FeSO₄ × 7H₂O | 0.01          | 0.01041         | 0.01063   |
| Na₂EDTA × 2H₂O | 0.08         | 0.0805          | 0.0805    |
| Trace metal solution | 1 mL | -           | -         |

| Component | Quantity used (g) |
|-----------|------------------|
|           | Distilled water | Stock solution (g L⁻¹) | Quantity used (to 1 L) |
| Na₂EDTA × 2H₂O | -             | 0.5 g                  |
| FeSO₄ × 7H₂O | -              | 0.7 g                  |
| ZnSO₄ × 7H₂O | 1.0           | 1 mL                   |
| MnSO₄ × 7H₂O | 2.0           | 1 mL                   |
| H₃BO₃      | 10.0           | 1 mL                   |
| Co(NO₃)₂ × 6H₂O | 1.0       | 1 mL                   |
| Na₂MoO₄ × 2H₂O | 1.0         | 1 mL                   |
| CuSO₄ × 5H₂O | 0.005         | 1 mL                   |

![Table 3: The composition of the Schlösser medium for *Spirulina* cultivation in different water included their elemental composition.](image)

![Table 4: Trace metals solution.](image)
stimated growth and achieved good quality of green pepper plants [58]. In another study, *Spirulina* suspension also helped to enhance the germination, plant growth and wheat (*Triticum aestivum* L.) grains (higher protein content). The concentration of *Spirulina* suspension was: 0.5, 1.0, 1.5, 2.0 and 2.5 g L⁻¹ respectively. The best effect was found for the dose of 1.5 g L⁻¹ of *Spirulina* suspension [57]. The *Spirulina* bio-product (2 g L⁻¹) was also tested in terms of growth, yield, nutritional value of seeds, chemical analysis of soil, nitrogen content of nodule and colony forming unit of cowpea (*Vigna unguiculata* (L.) Walp.) cultivated soil [20]. These microalgae increased cowpea growth, yield and protein content of seeds and were beneficial to the soil, as they enriched the soil microorganisms that help in recycling of organic nutrients (nitrogen, phosphorous and potassium).

These results confirm that *Spirulina* could be used as an organic fertilizer. Utilization of microalgae preparations in the production of commercial biofertilizers would fill the existing gap on the market. Properties of *Spirulina* products could be attributed to nutrients which are easily absorbed by plant leaves during the plant growth compared to the other treatment where soil fertilizers were added [58].

5 Conclusions

Due to the richness of nutrients in the biomass, *Spirulina* use in agriculture is becoming more popular. In Poland, the culture of microalgae in open systems in the winter is not possible. The solution to this problem could be the use of geothermal water and energy to prepare microalgal culture medium, heat greenhouses, which includes bioreactors, in order to maintain optimal temperature necessary for the growth of microalgae. Grown biomass could be used to produce biostimulants of plant growth and development. The performance of the preparations could be evaluated in the context of research carried out in plastic tunnels, using groundwater geothermal heating, which would allow the cultivation of plants in the winter. The use of geothermal water in cultivation of microalgae, heating of the cultivation water, soil and dryer with geothermal energy and the use of geothermal CO₂ can increase microalgal production and reduce its potential cost.

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