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Use of Trout Effluent as a Culture Medium for Growing *Spirulina platensis* Microalgae

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Abstract

Microalgae need rich sources of nitrate, phosphate, and potassium to grow, and fish farming effluents are rich in these compounds. An experiment was performed to investigate the effect of trout farming effluent as a culture medium on the growth and value of the main constituents of spirulina microalgae, including the value of protein, fat, and ash. According to the results, using trout farming effluent caused the growth and production of biomass in spirulina so that the value of dry biomass production was 0.3 g / l, and the value of protein, fat, and ash were 44, 0.9, and 13%, respectively. Although the number of spirulina trichomes in the treatment of fish farming effluent was less than the standard culture medium (control), this difference was insignificant on some days of the study so that on the ninth day of the experiment, the value of trichrome in ml was equal in both treatments. According to the research findings, trout effluent can be used as a cheap culture medium for growing spirulina.

Keywords: *Biomass, Biochemical compounds, Culture medium, Cyanobacteria, Wastewater.*

Introduction

Spirulina platensis microalgae is a species of blue-green algae from the cyanobacteria branch. Various species of this microalgae – that grow naturally in alkaline lakes and warm regions – have been used as food and dietary supplements for centuries in multiple communities (DiNicolantonio et al., 2020). Spirulina is seen as green spiral filaments with various diameters and lengths depending on the strain. Nowadays, spirulina microalgae are used as a feed supplement for fish, shrimp, and poultry (Moustafa et al., 2021, Rosenau et al., 2021).

Among its various species, "spirulina platensis" has received more attention given its nutritional compounds, including 55 to 75% of protein, mineral elements, vitamins, fatty acids and essential amino acids, antioxidants, and various pigments (Ramírez-Rodrigues et al., 2021). It is worth stating that spirulina microalgae and its extract are used in cosmetic and health products besides medicinal uses. It has been reported that spirulina microalgae account for about 30% of the global algal biomass production and is usually produced from culture media according to chemical compounds. In other words, this microalgae's cultivation environments need various compounds and chemical nutrients. Studies show that 35% of the production cost of this microalgae is spent on preparing its culture medium (Markou, 2021). Thus, using environments and compounds that can reduce this cost could be useful in reducing the production costs of this microalgae. Therefore, various types of chemicals, organic compounds, and alternative environments, especially effluents, have been studied and used to process spirulina microalgae (Lim et al., 2021).

Aquaculture cultivates different types of aquatic animals and plants in aquatic environments. Overall, one can state that aquaculture includes the breeding and production of edible, ornamental, medicinal, and industrial marine species in salty, semi-saline, and fresh waters. The industry is one of the simplest and most economical ways to produce animal protein. Aquatic protein is more valuable, functional, and digestible than other animal proteins. It must be stated that the value of aquaculture exploitation per unit area is usually more than agricultural products, and this issue doubles the significance of this field.

Based on the Food and Agriculture Organization (FAO) report, aquaculture is the fastest-growing industry and is always expanding (FAO, 2020). Currently, aquaculture industry products provide about half of the world's food. As aquaculture is an expanding activity, it has led to environmental problems like the destruction of marine habitats, chemical pollution, danger to biodiversity, reduction of immunological resistance of fish, the spread of diseases, mortality of aquatic organisms, and economic problems in the aquaculture industry (Naylor et al., 2021). Accordingly, taking measures to reduce these problems and the optimal use of the potential of this field seems necessary.

Water can be used in transit in many aquaculture activities. For instance, the water is used in transit for breeding cold-water fish like trout and can be used for other purposes such as agriculture after passing through the breeding channels. Such water is more suitable for agriculture due to the nutrients added to it through the excrement of fish or its excess nutrients. It has been reported that water from aquaculture farms has feces, uneaten food, and the remains of used drugs. Furthermore, it is estimated that fish absorb only 20 to 30% of nitrogen, and the rest is released into the water. Hence, besides suspended solids, a significant value of nitrogen and phosphorus is found in the effluent, the excessive accumulation of which causes toxicity to aquatic life. Nevertheless, it has been determined that microalgae could use the high nutrient and mineral compounds in the effluent to modify water pollution levels (Enwereuzoh et al., 2021).

In other words, using microalgae and cyanobacteria has recently been considered for purifying aquaculture effluents because of their growth capacity using solar energy and large values of nutrients (Tom et al., 2021). Microalgae can successfully absorb organic and mineral substances from the water environment and use these substances in the way of mass biomass production in light of their structure and special metabolic activities. Regarding this, some scholars have stated that using effluent associated with fish farming could not only be considered a suitable environment for the growth of spirulina but also these algae could bring the level of phosphate and ammonia in the water to the standard level and the water could be used aquaculture systems again (Wijayanti et al., 2019). It is worth stating that the biomass produced by microalgae has various biotechnological applications like the production of biofuels, biofertilizers in agriculture, aquatic feed, dye production, antioxidants, emulsifiers, enzymes, and so on (Nagappan et al., 2021. Fernández et al., 2021).

Given the above, using effluents from trout farming farms seems successful in cultivating spirulina microalgae. These effluents could bring about a cheap culture medium for cultivating this spirulina microalgae, which calls for more studies in this regard. Accordingly, this experiment was carried out to examine the use of trout effluent as a culture medium to cultivate spirulina microalgae.

Materials and methods

Algae preparation

The treated seed of spirulina platensis microalgae was supplied from the Sabzineh Zist Zagros Co. laboratory, and sea salt was added at the rate of 10 g/l for enrichment for mass cultivation to provide salinity and modified Jordan Culture Medium (Table 1) under 4000 lux of artificial light. Continuous aeration was used (Wijayanti et al., 2019). All the experiments were carried out in two treatments of trout effluent and filtered water with three repetitions to control the environmental conditions.

The effluent was collected from 2000-liter trout farming tanks with a density of 0.1 fish per liter after settling, which was fed using commercial foods containing 40% protein and 2800 kilocalories per kilogram.

Table 1. The components of the modified Jordan culture medium

Reagent	Concentration (g/l)
CH_4N_2O	0.07
$MgSO_4.7H_2O$	0.2
$FeSO_4.7H_2O$	0.005
NH_4HPO_4	0.1
K_2SO_4	1
KNO_3	2
NaCl	10
NaHCO3	8

It was sterilized with the help of an autoclave at 121°C for 15 min. A pressure of 1.5 atmospheres was maintained to eliminate the microbial contamination load and then used after cooling and adjusting the salinity. Microalgae spirulina was tested three times in the same temperature (29°C) and light (4000 lux) conditions, as 12 hours of light and 12 hours of darkness in 12 days to examine the growth of microalgae and its compounds, and was added to the effluent for growth. In this experiment, the specific growth rate (SGR) in the Batch system was obtained using the equation for various treatments.

$SGR= (LnN_2-LnN_1)/ \Delta t$

Where N_2 is the number of algae cells at the end of the experiment and N_1 is the number of algae cells at the start of the experiment, and Δt is the duration of the experiment.

Cellular density and optical density

Examining the growth of spirulina was carried out by daily measurement of absorption (optical density (OD)) of 0.025 liters of liquid culture using a spectrophotometer with a wavelength of 680 nm, and the following equation was used to obtain (linear regression). The correlation coefficient of the calibration curve was 0.97 (Wijayanti et al., 2019).

CD (Trichomes /ml) = [(OD+) / 0.127) 0.197]× 10

Determining and measuring spirulina product

Algae biomass was separated from the culture medium and dried using a 25-micron filter to measure the product production.

The analysis of spirulina chemical compounds and nutrients

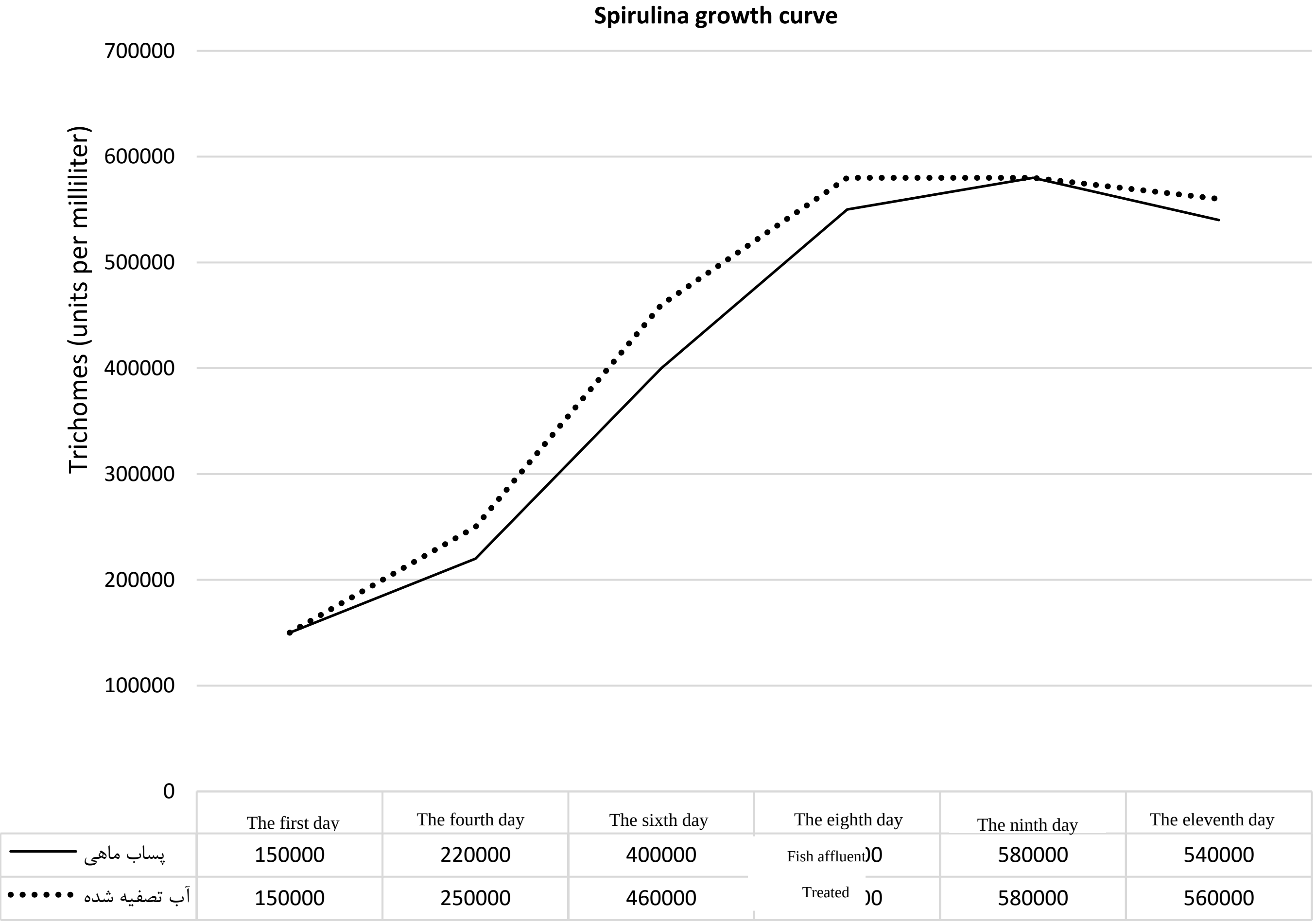
One gram of spirulina powder was placed in an oven at 105°C, and the weight difference was calculated within 3 hours to measure the moisture content of algae isolated from various treatments. One gram of spirulina was placed in the furnace at 625°C to measure the ash, and the weight difference was calculated when its color turned white.

To measure the protein, 0.3 grams of spirulina powder was put into the digester with concentrated sulfuric acid and Kendal tablets. Then uric acid was introduced into the nitrogen distillation apparatus, and the average titer and protein percentage were determined with hydrochloric acid 0.5 (Seghiri et al., 2019).

One gram of the sample was placed in a thimble and put into the Soxhlet device to extract fat by the Soxhlet method. The sample was washed with hexane several times, and the fat was extracted. Then the hexane was removed by rotary at a temperature of 45°C. At the end of the experiment, the results were analyzed by analysis of variance (ANOVA).

Results

Figure 1 shows spirulina's growth curve in various treatments in five phases. In the treatment of trout effluent, the culture started with an approximate average of 150,000 trichomes per milliliter and remained in the adaptation phase for two days. When its number reached about 220,000 trichomes per milliliter, it entered the growth phase, and the density of cells reached about 400,000 trichomes per milliliter (at the end of the stage on the fifth day); after that, the growth decreased and entered the stationary phase. The number of cells at the end of the phase was 550,000 trichomes per milliliter and then entered the death phase. In the control treatment (treated water with Jordan culture medium), microalgae growth started with an approximate average of 150,000 trichomes per milliliter. On the second day, the number of cells reached 250,000 trichomes per milliliter, and at the end of the phase, it reached 580,000 trichomes per milliliter (Figure 1). According to the results, the number of trichomes in the filtered water environment was more than that of the effluent of trout, so the number of trichomes in the filtered water was 12, 13.04 and 1.5% higher in the fourth, sixth, and eighth days, respectively. The number



	The first day	The fourth day	The sixth day	The eighth day	The ninth day	The eleventh day
پساب ماهی	150000	220000	400000	Fish affluent)0	580000	540000
آب تصفیه شده	150000	250000	460000	Treated)0	580000	560000

Figure 1. Spirulina growth curve and number of trichomes under trout effluent treatments and treated water

of trichomes in trout effluent and filtered water was the same on the ninth day.

The analysis of the main compounds of spirulina grown in Jordan and trout effluent culture media are listed in Table 2. One can see that the percentage of protein, fat, and ash of spirulina produced in the environment (treated water + Jordan) was higher than that produced in the culture environment from trout effluent. The value of spirulina protein, fat, and ash in the Jordan environment was 30, 25, and 23% higher than spirulina produced from trout effluent (Table 2).

Table 2: Analysis of the main compounds of spirulina cultivated in two culture environments (Jordan and trout effluent)

Table 2: Analysis of the main components of spirulina grown in two mediums (Jordan and trout effluents)

Ash (percentage)	Fat (percentage)	Protein (percentage)	Sample type
10	1.2	63	Spirulina (treated water + Jordan)
13	0.9	44	Spirulina (fish effluent)

Discussion

In this experiment, the number of trichomes in trout effluent and filtered water was the same on the ninth day with no differences. This means that the number of trichomes in the Jordan culture medium being equal to that of trout effluent on the ninth day can be promising for the high efficiency of using trout effluent in the cultivation of spirulina microalgae. Overall, using the Jordan culture medium for spirulina producers is associated with a high cost and must be renewed during production. It seems that the nutrients in trout effluent can partially provide the needs of spirulina microalgae.

On the other hand, spirulina could be used as a suitable solution to reduce the environmental effects of effluents. Overall, in aquaculture, nitrogen and phosphate are the main residues in water, which can cause water atrophication. Excessive accumulation and concentration of these substances can cause harmful and toxic effects on the environment and aquatic life, and they must always be kept within the legal limit. According to published reports and examinations, the effect of aerification could be minimized using some methods like sedimentation, the use of biofilters, and purification of phytoplanktons, and as a result, the environmental impacts of effluents can be reduced (Wijayanti et al., 2019; Hawrot-Paw et al., 2020). Another experiment found that spirulina algae could purify biorefinery pollutants such as phosphate and ammonia from water (Dewinta et al., 2020), which is in line with the result of the present study.

Using spirulina could result in the recovery of trout effluent based on the study findings. Moreover, spirulina biomass was created in this culture medium. It seems that the presence of the required spirulina in the effluent environment has caused the growth and production of spirulina biomass. Regarding this, some scholars have reported the useful and effective use of effluents associated with fish farms in spirulina production, which is consistent with the present research result (Vijayarasa et al., 2021; Mezzomo et al., 2010). In an experiment conducted for using catfish breeding effluent on the growth of spirulina microalgae, the best result in terms of the number of trichomes and dry weight of spirulina was obtained in the treatment using catfish breeding effluent + a certain value of fertilizer compounds and the treatment using fresh water + fertilizer compounds ranked next (Lesmana et al., 2019). Another study found that using trout effluent caused more growth of chlorella microalgae biomass (Hawrot-Paw et al., 2020). These scholars introduced the desired effluent as a suitable culture medium for growing chlorella. These scholars stated the proper value of mineral salts like nitrogen and phosphorus, and other nutrients in the water as waste materials of aquatic animals as a suitable source for the growth and reproduction of spirulina.

In another experiment, spirulina biomass production in the environments obtained from tilapia fish farming effluent was less compared to the Zarouk commercial culture environment (Vijayarasa et al., 2021), in line with the results of the present study. It must be noted that the value and type of effluents in the effluents vary according to the kind of water where it lives, which increases the necessity of conducting additional studies on the effect of various effluents on microalgae growth.

Overall, the biomass produced by spirulina could be used for different purposes like preparing feed for livestock, poultry, and aquatic animals. Moreover, it must be used as one of the main components in producing nutritional supplements for humans and other creatures. Given its protein, essential fatty acids, amino acids, vitamins, and mineral elements, spirulina is one of the complete compounds and food supplements (Moustafa et al., 2021). It must be noted that this biomass could be used for energy production purposes. This biomass and its compounds, such as pigments, polysaccharides and carotene, sterols, vitamins, and unsaturated fats, benefit from it in many industries like food, cosmetic and health industries, and pharmaceuticals (Rosenau et al., 2021).

Although the spirulina produced in the Jordan culture medium had a higher level of compounds like protein, fat, and ash in the present experiment, the high cost of preparing this culture medium for the growth of spirulina reduces the value of this superiority to some extent. It is worth stating that even the presence of 44% protein in spirulina produced in fish effluent culture environment enables the use of algal biomass in many products like aquatic feed for carnivorous and herbivorous fish, cosmetic and health products, and so on. In other words, the spirulina produced in the organic culture examined had a suitable nutritional value. Another experiment showed that using an organic culture medium produced from fish waste significantly increased the biochemical properties of spirulina microalgae (like protein and phycocyanin percentage) compared to spirulina produced in Zarouk commercial culture medium (Shanthi et al., 2021).

Conclusion

According to the results, spirulina seems to be a suitable alternative for wastewater treatment in integrated systems with water recirculation or in multi-product systems with fish or shrimp. It preserves water quality for aquatic animals and prevents the risk of eutrophication. On the other hand, as spirulina is very fast growing and could grow in organic environments without losing its nutritional value, and its biomass could be easily separated by filtration, one can produce commercial biomass of spirulina with appropriate values of protein, fat, and ash using this technique (Michael et al., 2019). Overall, it is necessary to cultivate a microalgae species in an organic environment, which does not lose its nutritional value and compounds besides a high growth rate, so compatibility with this method on larger scales should be considered. Spirulina can adapt to various salinities and grows very fast in alkaline environments, easing the cultivation process of this microalgae. This method of growing spirulina has several benefits: producing commercial biomass with appropriate nutritional value, reducing the environmental effects of aquaculture, removing pollutants from effluents, and reusing water because of biological treatment. Accordingly, trout effluent can be used as a low-cost culture medium to produce commercial spirulina microalgae if growth compatibility is examined on a larger scale.

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Conflict of interest

None

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Ethics statement

This study was approved by department of biochemistry of Isfahan Payame noor university

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