University of Sistan and Baluchestan

Chemical Process Design

Available online at http://cpd.usb.ac.ir/



Cultivating Spirulina in Waste Saltwater: A Sustainable Solution for High-Protein Biomass Production from Solar Still Brine Discharge

Babak Kamali Shahri, Mir Mohammad Khalilipour*, Hamid Moghaddam Deimeh

Department of Chemical Engineering, University of Sistan and Baluchestan, Zahedan 98164, Iran

ARTICLE INFO

Article history:
Received: 2024-02-03
Received in revised form: 2024-04-15
Accepted: 2024-05-24
Published online: 2024-05-26

Keywords:
Desalination brine; Microalgae cultivation; Solar still; Spirulina; Sustainable protein

DOI: *10.22111/cpd.2024.49883.1041*

ABSTRACT

This study explores the cultivation of Spirulina using desalination brine waste as a growth medium, offering a sustainable method to produce high-protein biomass. Spirulina, a filamentous blue-green alga, is rich in protein and essential nutrients, making it an ideal alternative to traditional protein sources. In this research, desalination brine waste was used, and its salt concentration was optimized to create a conducive environment for Spirulina cultivation. The algae were grown under controlled light and temperature conditions, yielding 2.5 grams of dried biomass per 2 liters with a protein content of approximately 60%, along with significant amounts of carbohydrates and essential minerals like potassium, calcium, and sodium. Structural analysis confirmed the healthy growth of Spirulina filaments, and elemental analysis indicated a rich nutritional profile. These results suggest that Spirulina cultivation using desalination waste is not only feasible but also presents a highly nutritious, economically viable, and sustainable alternative protein source, particularly for athletes and health-conscious consumers. Moreover, this method provides an innovative approach to recycling desalination waste, contributing to more sustainable aquaculture and food production systems.

1. Introduction

Protein deficiency is a pervasive issue affecting millions globally, particularly in regions where access to traditional protein sources such as meat, fish, and dairy is limited due to economic or environmental constraints. The global population, projected to reach 9.7 billion by 2050, poses significant challenges for the agricultural sector, which is already struggling to meet current food demands without exacerbating environmental degradation. As traditional agriculture faces mounting pressures from climate change, resource depletion, and land scarcity, the search for

Corresponding author. Tel.: +98 54 3113 2813.
E-mail address: a.khalilipour@eng.usb.ac.ir (M.M. Khalilipour)

alternative, sustainable protein sources has intensified. Among these alternatives, microbial proteins particularly single-cell proteins like Spirulina platensis have garnered considerable attention for their high nutritional value and environmental sustainability.

Spirulina is a filamentous, blue-green alga (cyanobacterium) that has been consumed for centuries, especially by indigenous peoples in Central America and Africa. It is known for its exceptional protein content—up to 70% by dry weight and a nutrient profile rich in vitamins, minerals, essential fatty acids, and antioxidants. Its wide range of applications spans the health food industry, aquaculture, animal feed, and even biotechnology, where it is used in biofuel production and wastewater treatment [1-3]. In aquaculture, Spirulina has been shown to enhance the growth, immunity, and survival rates of various aquatic species, including fish and shrimp, making it an invaluable resource for sustainable fish farming [4].Spirulina naturally thrives in alkaline, saline environments, which enables its cultivation in open systems, as it can tolerate conditions that inhibit other microorganisms [5]. This adaptability has led to its broad application in biotechnology, including its use in food and feed additives, pharmaceuticals, cosmetics, and scientific research [6]. However, successful large-scale cultivation requires favorable climatic conditions, such as consistent sunlight and stable temperatures. Environmental factors like sunlight intensity, rainfall, wind, and temperature fluctuations pose challenges to achieving optimal growth [7]. The cultivation of Spirulina also demands higher nutrient and salt concentrations than other algae like Sandamos and Chlora, a characteristic that may explain its natural abundance in salt lakes [8]. While diverse production methods exist, they are generally simplified approximations of the optimal environment first described by Zaruk. For ideal growth, Spirulina requires an alkaline environment with a constant supply of bicarbonate ions [9]. Research has shown that Spirulina cannot utilize organic compounds as its sole carbon source. Studies have found that sodium chloride-free media, supplemented with organic fertilizer extracts, can increase dry matter production by 5-30% in Spirulina subsalsa and other species [10].

Innovative approaches have expanded the applications of Spirulina, as exemplified by Fawad et al. [11], who developed an electrochemical sensor using Spirulina platensis coated with silver nanoparticles to evaluate atropine, an anticholinergic drug. This sensor demonstrated pH-dependent electrochemical behavior, stability, reproducibility, and selectivity, especially at pH 10. In another study, Tarangaraj et al. [12] conducted a comprehensive review of Spirulina production, marketing, and cost-benefit analysis, highlighting its potential as a single-cell protein source, especially for small-scale cultivation. The findings pointed to its utility in addressing malnutrition and enhancing livelihoods in developing nations like India, where commercial Spirulina cultivation presents a viable agribusiness opportunity.

Further research has focused on improving extraction techniques for valuable compounds within Spirulina. Rodrigues et al. [13] developed an ultrasonic-assisted extraction method for phycobiliproteins using protic ionic liquids, optimizing extraction parameters like pH and solvent-to-biomass ratio. Similarly, Su et al. [14] investigated the solid-liquid extraction of phycocyanin, establishing that the process followed second-order kinetics, with optimal extraction conditions identified within specific pH and temperature ranges. Wang et al. [15] improved phycobiliprotein extraction from dry Spirulina biomass by using sodium chloride, which enhanced cell disruption and extraction yield. Barmjo et al. [16] examined the antioxidant properties of phycocyanin, demonstrating its ability to scavenge reactive oxygen species and chelate metals, thereby suggesting its potential use in preventing free radical-related diseases. Matos et al. [17] explored the use of desalination concentrate as a medium for cultivating Spirulina. Their findings revealed that Spirulina grown in a 60% concentrate achieved a protein content comparable to other nutrient sources.

It supported the biosynthesis of carbohydrates, amino acids, fatty acids, and phycocyanin, which have potential applications as bio fertilizers and nutritional supplements.

In recent years, due to the water shortage crisis, extensive studies have been conducted on desalination of seawater and brackish water using solar stills [18-20]. However, few studies have studied the possibility of reusing the brine discharge of such devices. This study seeks to bridge that gap by investigating the cultivation of Spirulina in desalination brine waste. We aim to determine whether desalination waste, with its high salt content, can be optimized to support the growth of Spirulina and yield biomass rich in protein and essential nutrients. Our research also evaluates the potential of this process as a sustainable method for recycling desalination waste, contributing to a more circular economy.

2. Methodology

The experiment was designed to cultivate Spirulina using desalination brine waste under controlled laboratory conditions. The goal was to assess the viability of desalination brine as a medium for Spirulina growth. Their findings revealed that Spirulina grown in a 60% concentrate achieved by adjusting the salt concentrations and ensuring that environmental factors such as light and temperature were optimized for algal development.

2.1. Desalination unit and water preparation

The desalination unit utilized in this study was a stepped solar still, designed to efficiently produce fresh water [21] while generating brine waste for Spirulina cultivation. The system's construction material, aluminum, was selected for its excellent corrosion resistance at low and moderate temperatures, lightweight nature, and ease of fabrication.

Fig. 1(a) presents a cross-sectional view of the solar-stepped water still, which features a stepped absorber plate covered by inclined glazing, creating a 2.5cm gap that forms a collection chamber. The absorber plate comprises 15 steps, each measuring 60 cm in width, 5 cm in length, and 3 cm in height. This design provides an evaporation surface area of approximately 0.45 square meters. The step dimensions, with a length-to-height ratio of 5:3cm, are based on an optimized slope angle of 30 degrees, identified as ideal for the geographical location of Zahedan.

To regulate the flow of water across the absorber plate, each step is equipped with a 3 mm high, 59 cm long weir.

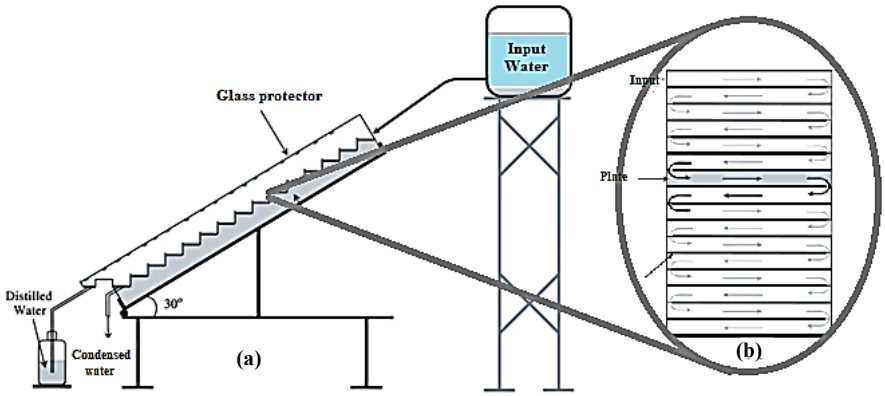


Fig. 1. (a) Cross-section of the solar stepped water solar still, (b) top view of the water flow path

Fig. 1(b) illustrates a schematic top view of the water flow path. Varying concentrations of salt solution were introduced into the water solar still, with inlet concentrations detailed in Table 1. After the desalination process, the

collected brine was characterized for total dissolved solids (TDS), *initially measuring 750-380 x 10*ppm. The desalination brine was then diluted with fresh water to adjust its TDS to the optimal range for Spirulina growth, which was determined to be 700 x 10 ppm. The prepared medium was placed in a 10-liter container, and 1 liter of Spirulina inoculum was introduced.

Table 1. Input concentration to Solar still in each sample

Sample number	Input concentration to Solar still
1	10×150 ppm
2	10×600 ppm
3	10×800 ppm
4	10×931 ppm
5	10×939 ppm
6	10×945 ppm
7	10×960 ppm
8	10×985 ppm

2.2. Cultivation conditions

To facilitate optimal growth, the Spirulina culture was aerated using an aquarium pump connected to an air stone. This ensured sufficient carbon dioxide delivering and mixing of the medium. The container was kept in a contamination-free environment throughout the process. The culture was exposed to a controlled light source (white light at a distance of 15-20cm) for 16 hours per day, followed by an 8-hour dark period, to simulate natural diurnal cycles. The temperature was maintained at 35°C, which is considered optimal for Spirulina growth.

The growth of Spirulina was monitored daily, and the culture was harvested after two weeks, when the algal biomass had reached to the maximum generation rate. After this days, the color of the culture medium is completely dark green. The algae were separated from the growth medium using a polyester fabric filter with a 30-micron pore size. The filtered biomass was then sun-dried and pulverized into powder form for further analysis. A schematic of the cultivation process is presented in Fig. 2.

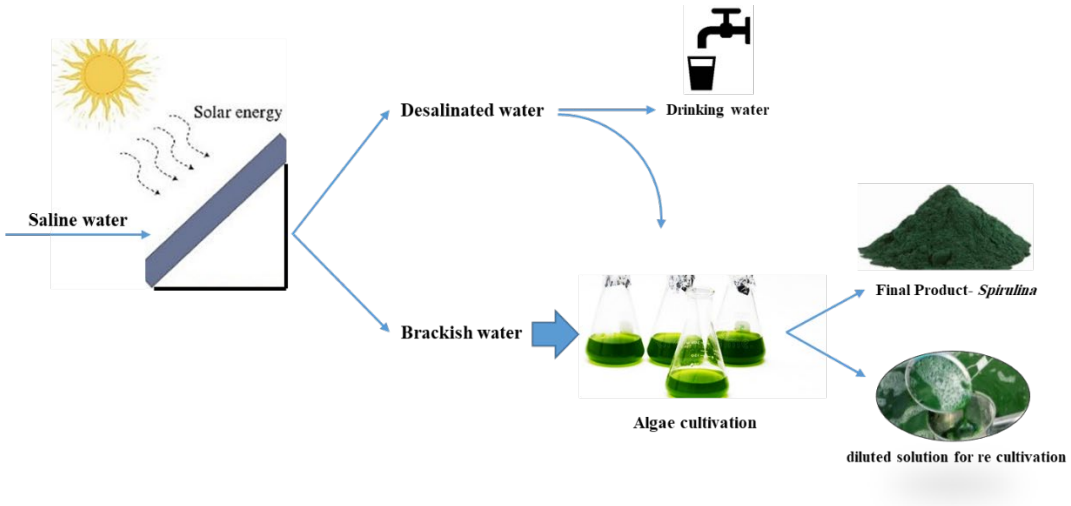


Fig. 2. A schematic of the cultivation process

2.3. Analytical procedures

The dried Spirulina biomass was analyzed for its macronutrient content, including protein, carbohydrates, fats, and moisture. Elemental analysis was conducted to determine the concentration of essential minerals and trace elements,

using a sixty-element test. Structural and microstructural analyses of the Spirulina filaments were carried out using optical microscopy to evaluate the quality of the cultivated algae and confirm the absence of contamination from other microorganisms.

3. Results

3.1. Water and salt concentration adjustments

The TDS (Total Dissolved Solids) of the desalination brine was adjusted to 700 x 10 ppm, which created a highly conducive environment for Spirulina cultivation. This carefully calibrated medium, further enriched with a balanced fertilizer stock, ensured optimal conditions for robust algal growth. Following Spirulina inoculation, the medium's hardness increased significantly, reaching a value of 16,000 ppm. This adjustment highlights the efficacy of the brine in supporting Spirulina's physiological needs, particularly in high-salinity conditions, making it a viable growth medium.

3.2. Biomass yield and growth observations

After two weeks of cultivation, 50 grams of dried Spirulina biomass was successfully harvested. This yield aligns with existing studies on Spirulina cultivation under controlled conditions and can be considered satisfactory given the experimental parameters. The Spirulina filaments, when observed through optical microscopy, exhibited healthy growth patterns, with their lengths ranging from 200 to 500 microns, confirming the appropriateness of the cultivation conditions (Fig. 3). The absence of contamination from other algae or microorganisms further validates the precision of the cultivation protocol and the suitability of desalination brine as a growth medium. Such growth is indicative of a well-maintained environment that supports Spirulina’s natural physiology, enhancing both yield and quality.

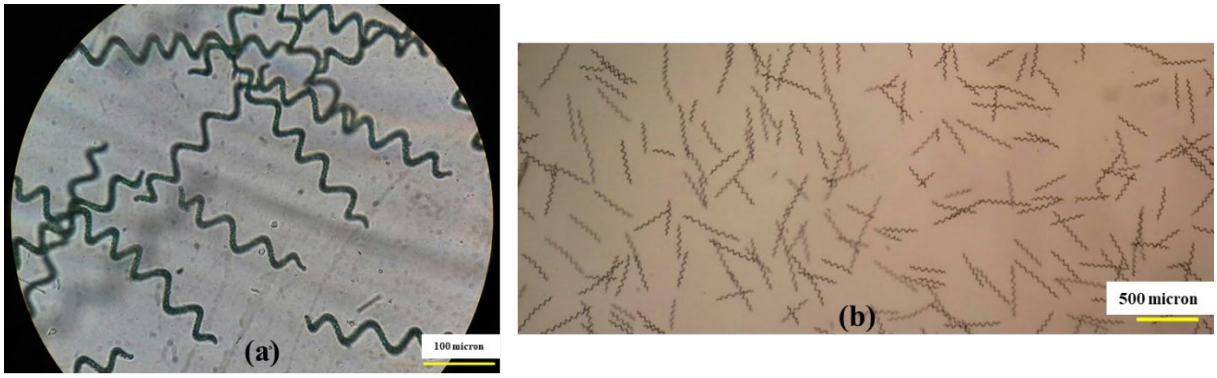


Fig. 3. Spirulina algae filaments grown at two magnifications, (a) 100 micron and (b) 500 microns

3.3. Elemental analysis

A comprehensive 60-element analysis was conducted to determine the elemental composition of the cultivated Spirulina. The results, as presented in Table 2, indicate that the Spirulina biomass is rich in essential minerals such as potassium (K), phosphorus (P), calcium (Ca), manganese (Mn), sodium (Na), and sulfur (S), all of which play crucial roles in human nutrition and Spirulina’s metabolic processes. Additionally, the presence of significant quantities of iron (Fe) and trace amounts of gallium (Ga) suggests the biomass could have further health benefits. The diversity of elements detected, including those critical for biological functions, underscores Spirulina's value as a nutritionally dense food source.

Table 2. The sixty-element analysis of the Spirulina biomass

Element	Ag	Al	As	B	Ba	Be	Bi
ppm	<0.5	39.16	<0.5	35.4	10.37	<0.5	<0.5
Element	Ca	Cd	Ce	Co	Cr	Cs	Cu
ppm	3646.02	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Element	Dy	Er	Eu	Fe	Ga	Gd	Ge
ppm	<0.5	<0.5	<0.5	235.40	2.99	<0.5	<0.5
Element	Hf	Hg	Ho	In	Ir	K	La
ppm	<0.5	<0.5	<0.5	<0.5	<0.5	>10000	<0.5
Element	Lu	Mg	Mn	Mo	Na	Nb	Nd
ppm	<0.5	<0.5	3654.84	9.2	3601.77	<0.5	<0.5
Element	Os	P	Pb	Pd	Pr	Pt	Re
ppm	<0.5	>10000	1.53	<0.5	<0.5	<0.5	<0.5
Element	Rh	Ru	S	Sb	Sc	Se	Sm
ppm	<0.5	<0.5	2893.81	<0.5	<0.5	<0.5	<0.5
Element	Sn	Sr	Ta	Tb	Te	Th	Ti
ppm	<0.5	44.25	<0.5	<0.5	<0.5	<0.5	<0.5
Element	Tl	Tm	U	V	W	Yb	Zn
ppm	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	48.94

3.4. Nutritional composition

Macronutrient analysis of the produced Spirulina biomass is presented in Table 3. The macronutrient profile of the Spirulina biomass revealed that it contained approximately 60% protein, which confirms its status as a high-protein biomass. The carbohydrate content was measured at 22%, while the fat content was notably low at 1.67%, maintaining Spirulina's reputation for being a low-fat, nutrient-rich food. These findings align with previously documented nutritional profiles of Spirulina, reinforcing its potential as a dietary supplement. The moisture and ash content were also recorded at 10.5% and 6.5%, respectively, contributing to the overall understanding of the biomass's composition and stability. This composition makes Spirulina an attractive option for those seeking a natural, plant-based source of protein and essential nutrients.

Table 3. Macronutrient analysis of the Spirulina biomass

Test	Value (Weight percent)	
	Dairy coefficient	Meat coefficient
Protein	60.18	58.95
Carbohydrate	21.1	22.33
Moisture	10.5	
Ash	6.53	
Fat	1.67	

4. Discussion

This study demonstrates the feasibility of using desalination brine waste as a medium for Spirulina cultivation, presenting a novel approach to sustainable aquaculture and waste management. The Spirulina grown in desalination brine exhibited healthy growth characteristics, with filaments reaching optimal lengths and no visible contamination from other microorganisms. The 2.5 grams of dried biomass per 2 liters from a small-scale cultivation setup suggests that this method can be scaled up for larger production, especially in regions where desalination is common and freshwater resources are scarce.

The nutritional analysis of the Spirulina biomass revealed a high protein content, comparable to commercial protein supplements like whey protein concentrate, which typically contains 60-80% protein by weight. Spirulina's high protein content, coupled with its rich mineral composition, makes it an excellent candidate for use as a dietary supplement, particularly for athletes seeking plant-based protein sources. Utilizing desalination brine not only

addresses the issue of saline waste disposal but also contributes to a more circular economy by transforming waste into a valuable resource. This approach aligns with global sustainability goals, promoting the use of renewable resources and reducing the environmental impact of food production. Furthermore, this study underscores the importance of innovative solutions for food security, particularly in arid regions where water scarcity poses significant challenges to traditional agriculture.

Future research should focus on optimizing cultivation parameters, including light intensity and nutrient supplementation, to enhance biomass yield and nutrient density further. Additionally, investigating the economic viability of large-scale Spirulina production using desalination brine could provide valuable insights into the potential for commercial applications.

5. Conclusion

In conclusion, this study highlights the potential of Spirulina cultivation using desalination brine waste as an innovative and sustainable method for protein production. The successful growth of Spirulina in high-salinity conditions underscores its adaptability and resilience as a protein source. With a protein content of approximately 60% and a rich array of essential nutrients, Spirulina offers a promising alternative for addressing global protein deficiencies while also contributing to sustainable waste management practices.

Reference

[1] Bădescu, V., 1985. The influence of certain astronomical and constructive parameters on the concentration of solar radiation with plane heliostats fields. *Revue de physique appliquée*, 20(10), 711-733.
[2] Dorian, M.E., Nelson, D.H., 1980. Solar tracking device. U.S. Patent 4,223,214.
[3] Yilmaz, S., Ozcalik, H.R., Dogmus, O., Dincer, F., Akgol, O., Karaaslan, M., 2015. Design of two axes sun tracking controller with analytically solar radiation calculations. *Renewable and Sustainable Energy Reviews*, 43, 997-1005. http://dx.doi.org/10.1016/j.rser.2014.11.050
[4] Nsengiyumva, W., Chen, S.G., Hu, L., Chen, X., 2018. Recent advancements and challenges in Solar Tracking Systems (STS): A review. *Renewable and Sustainable Energy Reviews*, 81, 250-279. http://dx.doi.org/10.1016/j.rser.2017.06.085
[5] Eldin, S.S., Abd-Elhady, M.S., Kandil, H.A., 2016. Feasibility of solar tracking systems for PV panels in hot and cold regions. *Renewable Energy*, 85, 228-233. http://dx.doi.org/10.1016/j.renene.2015.06.052
[6] Kvasznicza, Z., Elmer, G., 2006. Optimizing solar tracking systems for solar cells. In *Proceedings of the 4th Serbian–Hungarian Joint Symposium on Intelligent Systems*, September, Subotica, Serbia, 77-84.
[7] Pelizer, L.H., Danesi, E.D.G., de O Rangel, C., Sassano, C.E., Carvalho, J.C.M., Sato, S., Moraes, I.O., 2003. Influence of inoculum age and concentration in *Spirulina platensis* cultivation. *Journal of Food Engineering*, 56(4), 371-375. http://dx.doi.org/10.1016/S0260-8774(02)00268-4
[8] Kawata, Y., Yano, S.I., Kojima, H., Toyomizu, M., 2004. Transformation of *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC9438) with Tn5 transposase–transposon DNA–cation liposome complex. *Marine Biotechnology*, 6, 355-363. http://dx.doi.org/10.1007/s10126-004-3056-7
[9] Volkmann, H., Imianovsky, U., Oliveira, J.L., Sant'Anna, E.S., 2008. Cultivation of *Arthrospira* (*Spirulina*) platensis in desalinator wastewater and salinated synthetic medium: Protein content and amino-acid profile. *Brazilian Journal of Microbiology*, 39, 98-101. http://dx.doi.org/10.1590/S1517-83822008000100022
[10] Vonshak, A., ed., 1997. Spirulina platensis arthrospira: Physiology, Cell-Biology and Biotechnology. CRC Press.
[11] Venkataraman, L.V., Sindhu Kanya, T.C., 1981. Insect contamination in the mass outdoor cultures of blue green alga, Spirulina platensis. In Proceedings of the Indian Academy of Sciences, 90(6), 665-672.
[12] Thangaraj, R., Mahendran, S., Nizhanthini, C., Dhanasekaran, D., Thajuddin, N., 2023. Small/Large-Scale Production, Cost Benefit Analysis, and Marketing of Spirulina Single Cell Protein. In Food Microbiology Based Entrepreneurship: Making Money from Microbes, Springer Nature Singapore, 115-132.
[13] Rodrigues, R.D.P., de Castro, F.C., de Santiago-Aguiar, R.S., Rocha, M.V.P., 2018. Ultrasound-assisted extraction of phycobiliproteins from Spirulina (Arthrospira) platensis using protic ionic liquids as solvent. Algal Research, 31, 454-462. http://dx.doi.org/10.1016/j.algal.2018.02.016
[14] Su, C.H., Liu, C.S., Yang, P.C., Syu, K.S., Chiuh, C.C., 2014. Solid–liquid extraction of phycocyanin from Spirulina platensis: Kinetic modeling of influential factors. Separation and Purification Technology, 123, 64-68. http://dx.doi.org/10.1016/j.seppur.2013.12.019

[15] Wang, F., Yu, X., Cui, Y., Xu, L., Huo, S., Ding, Z., Hu, Q., Xie, W., Xiao, H., Zhang, D., 2023. Efficient extraction of phycobiliproteins from dry biomass of Spirulina platensis using sodium chloride as extraction enhancer. Food Chemistry, 406, 135005. http://dx.doi.org/10.1016/j.foodchem.2023.135005
[16] Bermejo, P., Piñero, E., Villar, Á.M., 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulina platensis. Food Chemistry, 110(2), 436-445. http://dx.doi.org/10.1016/j.foodchem.2008.02.065
[17] Matos, A.P., da Silva, T., Sant'Anna, E.S., 2021. The feasibility of using inland desalination concentrate (DC) as an alternative substrate for Spirulina platensis mass cultivation. Waste and Biomass Valorization, 12, 3193-3203. http://dx.doi.org/10.1007/s12649-020-01291-7
[18] Samimi, M., Moghadam, H., 2024. Modified evacuated tube collector basin solar still for optimal desalination of reverse osmosis concentrate. Energy, 289, 129983. http://dx.doi.org/10.1016/j.energy.2023.129983
[19] Samimi, M., Moghadam, H., 2024. Investigation of structural parameters for inclined weir-type solar stills. Renewable and Sustainable Energy Reviews, 190, 113969. http://dx.doi.org/10.1016/j.rser.2023.113969
[20] Abrofarakh, M., Moghadam, H., Abdulrahim, H.K., 2024. Investigation of direct contact membrane distillation (DCMD) performance using CFD and Machine Learning approaches. Chemosphere, 141969. http://dx.doi.org/10.1016/j.chemosphere.2024.141969
[21] Tabrizi, F.F., Dashtban, M., Moghaddam, H., Razzaghi, K., 2010. Effect of water flow rate on internal heat and mass transfer and daily productivity of a weir-type cascade solar still. Desalination, 260(1-3), 239-247. http://dx.doi.org/10.1016/j.desal.2010.03.037