

Characterization of *Arthrospira* sp (Spirulina) Biomass Growth in Hydroponic Waste Solution: A Review

Yan Valdez Santos Rodrigues^{1*}, Edna Dos Santos Almeida², Erika Durão Vieira³

¹SENAI CIMATEC University Center, Biotechnology Laboratory; ²SENAI CIMATEC University Center, Department of Environment; ³SENAI CIMATEC University Center, Biotechnology Laboratory; Salvador, Bahia, Brazil

The use of *Spirulina* (*Arthrospira* sp) as a food source has stood out for being a potential source of proteins, fatty acids, vitamins, and others. However, the cultivation medium for its production is relatively expensive. Then, an alternative culture medium is necessary for reducing costs. So, hydroponics drainage effluent, a solution rich in nutrients, represents an alternative source of substrate for microorganism cultivation. Thus, this work aimed to characterize the biomass of *Arthrospira* sp grown in an alternative culture medium based on hydroponic effluent through a systematic review. We noted a trend to reduce biomass growth and bioactive compounds due to an excess of iron and copper in the effluent. The study concluded that it needs to supplement the residual medium with a synthetic medium for its use to obtain biomass for commercial utilization. **Keywords:** *Arthrospira* sp. Hydroponic Waste. Drainage Medium. Biomass Composition. Microalgae.

Introduction

The use of microalgae and cyanobacteria as a food source, both for humans and animals, has stood out for being a potential source of proteins, unsaturated fatty acids, vitamins, minerals, pigments, enzymes, antibiotics, and other biologically active metabolites [1,2]. However, the potential associated with these microorganisms depends directly on the variables related to the growth of biomass. Therefore, it is necessary to control the pH, light, temperature, and nutrients. Nutrients are supplied to the microorganism by a solution containing nitrogen, phosphorus, potassium, some salts, and metals in lower concentrations, which is designated as a culture medium and represents the most expensive cost for the production of biomass [3].

The hydroponics technique uses a nutrient solution that feeds the plants replacing the soil.

However, this solution discarded periodically, generating a drainage solution rich in nutrients [4].

Spirulina (*Arthrospira* sp) is the cyanobacteria (blue-green alga) most used in animal and human food due to the high protein content in biomass (up to 60% of dry weight) and the high nutritional value associated with vitamins, minerals, and acids fatty content, biological activity, and easy digestion [5,6]. This algae species has been approved by ANVISA (Brazilian Health Surveillance Agency) for human consumption. Due to the existence of different *Spirulina* strains, they can adapt to extreme environments (alkaline environments, saline environments, high temperatures, and light, among others), and their cultivation and consumption have popularized in many parts of the world [5]. Thus, an alternative medium based on hydroponic waste for the cultivation of cyanobacteria *Arthrospira* sp becomes an interesting opportunity to save costs and avoid wastewater disposal.

The objective of this work was to determine the proximate profile (carbohydrate, protein, lipids) of the biomass of *Arthrospira* sp cultivated in alternative culture media based on hydroponic effluent through a systematic review to identify the effects of low-cost culture media on biomass composition.

Received on 5 September 2020; revised 17 September 2020.
Address for correspondence: Yan Valdez Santos Rodrigues,
SENAI CIMATEC, University Center, Biotechnology
Laboratory, Av. Orlando Gomes, Piatã, Número 1845, Zip
Code: 41650-010, Salvador, BA, Brazil, Phone/Fax: +55 (71)
3534-8090. E-mail: yanvaldez@gmail.com.

J Bioeng. Tech. Appl. Health 2020;3(4):354-358.
© 2020 by SENAI CIMATEC. All rights reserved.

Materials and Methods

The keywords used for the research in this study as follow: Microalgae, hydroponics, aquaponics, tomatoes, comparison, alternative media, cultivate media, NPK, Spirulina, Biomass composition, *Arthrospira*, aquaculture, by which 78 combinations were made. The scientific database used was ScienceDirect with no limitation on publication time and restricted to individual articles. The keywords were searched both in the title field and within the entire text. A total of 49 articles were found on the ScienceDirect search platform considering the following exclusion criteria:

- No publications with not present full articles available;
- Adherence to the objectives of informational research.

Results and Discussion

After applying systematic review criteria, 19 articles were selected, and part of these articles was used to characterize the proximate profile of *Arthrospira* sp biomass. Evaluating the articles, studies that use the exactly hydroponic wastewater for *Arthrospira plantensis* or *Arthrospira* sp cultivation was no found in this search.

The studies found that uses culture media with a proximate composition to the hydroponics or/and aquaponic effluent for *Arthrospira* sp cultivation were those based on aquaculture residues (Table 1) [7,8]. Thus, we included other microalgae species, specifically *Chlorella* sp in the search, because was reported in the literature the cultivation of this species with the hydroponics drainage effluent as a growing medium (Table 2).

Analyzing the effects of these alternative culture media in the growth of microalgae with studies carried out for *Arthrospira* sp and observing the similarities and differences in the effect on the biomass composition (in terms of carbohydrate, protein, and lipids profile), it was identified some points to serve as arguments in the Spirulina

biomass characterization cultivated in a residual hydroponic medium.

All experiments involving the hydroponic and aquaculture wastewater founded excellent results for the use of residual effluents. However, there is a need to complement this solution with other micronutrients to obtain good results in the production of biomass and by-products of high added value. Also, in some of the selected works, the use of a pre-treatment process of residual effluent was observed to eliminate the presence of microorganisms that could negatively influence the microalgae development and also reduce or increase micronutrient levels, which may have inhibitory or catalytic effects.

Thus, the high presence of metals like Fe and Cu, reduction or excess of essential nutrients, and other events can interfere in the growth of *Spirulina* through alterations in the metabolism or reducing its activity [2, 7, 15].

After the evaluation of each selected work, it was possible to verify the approximate profile (carbohydrates, proteins, lipids) and compounds with high added value with reduced productivity expectation if used raw residual effluent. However, if this effluent is supplemented or treated for contaminants removal, a yield better or equivalent to that of synthetic culture media such as Zarrouk's solution can be obtained [16].

Besides, it is possible to infer that the biomass composition of *Arthrospira* sp cultivated in hydroponic effluent will be similar to that reported by Bertoldi and colleagues [1], who studied the cultivation of *Chlorella* sp in this alternative medium. The same inference could be made related to the results obtained by Cardoso and colleagues [8], who evaluated the growth of *Spirulina* in aquaculture wastewater medium. In both studies, we noticed that biomass with a similar composition to that observed for cultivation in the Zarrouk medium can be obtained.

Hultberg and colleagues [4] reported a normal or subpar amount of carotenoids, bioactive with high added value, depending on stress conditions and hydroponic waste with a possible reduction

Table 1. Studies that present alternative media for Arthrospira growth based on hydroponic, aquaponic, or aquaculture cultivation residues.

Ref.	Culture Medium	Main Results
[7]	Aquaculture Wastewater	The algae grew well in fish water with a specific growth rate of 0.026 h^{-1} (0.623 day^{-1}) and a doubling time of 28 h. These growth parameters compare favorably with those reported in the literature, indicating the adequacy of the cultivation of <i>S. platensis</i> in fish waters.
[8]	Aquaculture Wastewater	The best results of <i>Spirulina</i> sp LEB18 growth parameters - crops that used wastewater aquaculture - were obtained in experiments with 25% and 50% Zarrouk supplementation. In these experiments, the maximum biomass concentrations were equal ($p < 0.05$) to the control test without the addition of residual aquaculture water. Also, the highest yield rates and the maximum growth coefficients in these two experiments were about 1.7 and 1.8 higher, respectively, than the results obtained in the control cultivation. Although, the experiments using 75% Zarrouk supplementation showed a lower maximum biomass concentration than the control. The fatty acid composition of the biomass in the control, and with 25% supplementation of Zarrouk medium was observed in both experiments with the same profile. The content of unsaturated fatty acids in the treatment with 25% Zarrouk was high.
[9]	Confectionary Wastes	In this study, <i>A. platensis</i> grew very well in the residual confectionery effluent during the different experimental runs of the 22 CCD (Central Composite Design). The seaweed can use sugar present in the effluent. As expected, the results ensured the adequacy of the use of <i>A. platensis</i> for growth in residual confectionery effluent. Besides, the biochemical composition of the biomass was 20% higher than the control after 12 days. α , β carotenes (mg L^{-1}) was doubled. The levels of proteins, lipids, and carbohydrates increased by 20%, 22.2%, and 22.3%, respectively. However, chlorophyll decreased by 12% as the biomass became pale and lost its typical blue-green color.
[10]	Zarrouk Synthetic Medium with Added Glycerol	The addition of glycerol to <i>Spirulina</i> sp (LEB 18) stimulated cell growth (3.00 g L^{-1} of biomass, $0.72 \text{ g L}^{-1} \text{ d}^{-1}$ of maximum productivity) and protein production (69.78% w w-1), obtaining the best results at 0.05 mol L^{-1} . The substrate application had a significant effect on the composition of fatty acids. The increase in unsaturated fatty acids, mainly oleic acid, and the reduction in saturated fatty acids, principally palmitic, were proportional to the increasing in the concentration of glycerol in this ambient.
[11]	Formulation of a Low-Cost Medium	This investigation was carried out with the principal objective of providing a simple and inexpensive environment, and our results indicate that the newly formulated solution RM6 is on the same level as Zarrouk's solution about the performance of <i>Spirulina</i> sp when evaluated in terms of chlorophyll, protein, or dry biomass.
[12]	Dairy Farm	Wastewater can support the growth of <i>Arthrospira platensis</i> for the production of biodiesel. <i>A. platensis</i> shows a wide range of temperature tolerance; however, 30° C was considered more suitable for the production of lipids. Change in light intensity from 160 to $300 \mu\text{mol} / \text{m}^2 / \text{s}$ during the intermediate exponential phase favors the production of saturated fatty acid in <i>Arthrospira platensis</i> .

Table 2. Studies that present alternative media for *Chlorella* growth based on hydroponic, aquaponic, or aquaculture cultivation residues.

Ref.	Species	Culture Medium	Main Results
[1]	<i>Chlorella vulgaris</i>	Residual Hydroponic Solution	The cell density in the cultivation of the residual hydroponic solution with 25% residue and 75% deionized did not present any significant difference about the control (BBM). The other crops (SHR and SHR50) were significantly lower than the control crop but did not show any statistical difference between them. The cultivation of <i>Chlorella vulgaris</i> in residual hydroponic solution represents an attractive option for the development of nutritional supplements due to the high protein content and biologically active substances present in the cell biomass obtained in the process. The residual hydroponic solution and its respective dilutions (SHR50 and SHR25) proved to be a good option as an alternative way of the environment in the cultivation of <i>Chlorella vulgaris</i> , enabling the recycling of this residue sustainably.
[4]	<i>Chlorella vulgaris</i>	Artificial Drainage Solution and Spent Drainage Solution*	This study showed that microalgae have the potential to reduce nutrient concentrations in the production drainage solution in a hydroponic greenhouse. We observed significant reductions in nitrogen and, in particular, in phosphorus concentration.
[13]	<i>Chlorella</i> spp	Co-Cultivation with Tomato in a Hydroponic System	In the EHS (eco-hydroponic culture), the production of microalgal biomass and the production of agricultural biomass was increased by aerating, by the photosynthesis of the algae, and by fertilizing C of the crop root respiration and exudation, respectively. In particular, the co-use of nutrients in the nutrient solution by microalgae and culture can increase the efficiency of nutrient use and minimize the discharge of nutrients.
[14]	<i>Platymonas subcordiformis</i>	Aquaculture Wastewater	It was possible to couple the removal of nitrogen and phosphorus from wastewater to algae biomass and the production of biofuels. <i>Platymonas subcordiformis</i> kelp can remove 87%-95% nitrogen and 98%-99% phosphorus in sole aquaculture wastewater. The algae biomass was 8.9 times greater than the initial level.

*Solution used in commercial greenhouse cultivation of tomato.

of up to 50% of these substances, considering the case of the wastewater having an excess of metallic compounds such as Fe, Cu, and Mo, there may be a reduction in biomass and proteins [15,17].

Conclusion

We observed that all experiments involving the hydroponic and aquaculture wastewater as culture media founded optimistic results for the use of residual effluents enriched with other synthetic culture media. A trend to reduce biomass production and bioactive compounds mainly when the microorganism suffers the effects of the excess of some micronutrients such as iron and copper, which is associated with the use of wastewater was observed.

Thus, analyzing the articles makes it possible to infer that the biomass of *Arthrospira* sp will be composed of biomass with protein yield and concentration of carotenoids similar to the standard Zarrouk medium if the effluent is added as a complement in the culture medium. This study brought some insights from literature about the feasibility of the use of hydroponic, aquaponic, or aquaculture cultivation.

Acknowledgments

We are grateful FAPESB for the scholarship granted under the project PRODUCTION OF MICRO-ALGAE FOR FOOD PURPOSES USING EFFLUENT FROM HYDROPONIC CULTIVATION OF TOMATO, which was approved in application N° 3497/2019.

References

- Bertoldi FC, Sant'anna E, Oliveira JLB. Teor de clorofila e perfil de sais minerais de *Chlorella vulgaris* cultivada em solução hidropônica residual. *Ciência Rural* 2008;38:54–58.
- Soni RA, Sudhakar K, Rana RS. Spirulina – From growth to nutritional product: A review. *Trends in Food Science & Technology* 2017;69:157–171.
- Morais Junior WG. et al. Microalgae for biotechnological applications: Cultivation, harvesting and biomass processing. *Aquaculture* 2020;528:55–62.
- Hultberg M, Carlsson AS, Gustafsson S. Treatment of drainage solution from hydroponic greenhouse production with microalgae. *Bioresource Technology* 2013;136:401–406.
- Costa JAV. et al. Operational and economic aspects of Spirulina-based biorefinery. *Bioresource Technology* 2019;292:12–19.
- Chamorro-Cevallos G. Aspectos nutricionales y toxicológicos de spirulina (*Arthrospira*). *Nutricion hospitalaria* 2015;1:34–40.
- Wuang SC. et al. Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. *Algal Research* 2016;15:59–64.
- Cardoso LG. et al. Spirulina sp LEB 18 cultivation in outdoor pilot scale using aquaculture wastewater: High biomass, carotenoid, lipid and carbohydrate production. *Aquaculture* 2020;525:52–72.
- El-kassas HY, Heneash AMM, Hussein NR. Cultivation of *Arthrospira* (*Spirulina*) *platensis* using confectionary wastes for aquaculture feeding. *Journal of Genetic Engineering and Biotechnology* 2015;13:145–155.
- Morais EG. et al. Glycerol increases growth, protein production and alters the fatty acids profile of Spirulina (*Arthrospira*) sp LEB 18. *Process Biochemistry* 2019;76:40–45.
- Raof B, Kaushik BD, Prasanna R. Formulation of a low-cost medium for mass production of Spirulina. *Biomass and Bioenergy* 2006;30:537–542.
- Hena S. et al. Dairy farm wastewater treatment and lipid accumulation by *Arthrospira platensis*. *Water Research* 2018;128:267–277.
- Zhang J, Wang X, Zhou Q. Co-cultivation of *Chlorella* spp and tomato in a hydroponic system. *Biomass and Bioenergy* 2017;97:132–138.
- Guo Z. et al. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. *Journal of Environmental Sciences* 2013;25:85–88.
- Zinicovscaia I. et al. Metal ions removal from different type of industrial effluents using *Spirulina platensis* biomass. *International Journal of Phytoremediation* 2019;21:1442–1448.
- Zarrouk C. Contribution a l'etuded'unecyanophycee: influence de divers facteurs physiques etchimiquessur la croissance et la photosynthese de *Spirulina maxima* (Setch et Gardner) Geitler. *Theises. Faculty of Science. Universite des Paris*; 1966.
- Ismail MMS, Piercey-Normore MD, Rampitsch C. Proteomic analyses of the cyanobacterium *Arthrospira* (*Spirulina*) *platensis* under iron and salinity stress. *Environmental and Experimental Botany* 2018;147:63–74.