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Fisheries Wastewater as a Sustainable Media for the Production of Algae-Based Products

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Colombian intensive fish production is concentrated mainly in the departments of the Andean Region, Amazon, and Orinoquía. These systems were characterized for being exploited mainly by family farming nuclei, which are dedicated exclusively to breeding and others with mixed systems. Currently, the sustainable development of this economic line depends on two factors: global warming and the consumption of resources (energy, fresh water, and protein). The rapid growth of this socio-economic line has led to the development of 3 critical restrictions: the demand for food for fish production, the high volume of fresh water needed and the high concentration of wastewater which must be disposed of safely. Sewage from closed fish farming systems has high levels of nitrogen and inorganic phosphorus dissolved in the systems. The primary responsibility for these high contents is the feed which contributes to the sustained increase in the concentration of organic waste and toxic compounds in aquatic systems. To make use of this wastewater, the use of these as a culture medium for microalgal production has been studied in order to generate metabolites of industrial interest from a low-cost culture medium.
In this work, the necessary culture conditions for the biomass production of *Scenedesmus obliquus*, *Chlorella vulgaris*, *Spirulina maxima,* and *Oscillatoria sp.* in fish farming wastewater to produce pigments and total biomass are evaluated. The wastewater was obtained from an intensive fish farming company in El Zulia (Norte de Santander, Colombia). The medium was UV-sterilized (4 Lamps of 15W, 5 minutes). In order to optimize the production of biomass and pigments, the wastewater was adjusted with the addition of nitrogen, phosphorus, and carbon (K_2HPO_4 + $NaNO_3$ + $NaHCO_3$)
According to the results, the residual water enriched with K_2HPO_4, $NaNO_3$ and $NaHCO_3$ presented the best culture conditions for obtaining carotenoids (in *C. vulgaris* and *S. obliquus* with values of 2.6 and 1.7% p/p respectively) and Phycobiliproteins in *Spirulina maxima* and *Oscillatoria sp* (10.9 and 11% p/p respectively). These results allow concluding that the residual water of fish systems is outlined as a suitable culture medium that can be used to produce metabolites of interest. Also, this culture medium must be enriched in order to increase the productivity of the system.

1. Introduction

Since 1973, the world consumption of fish has doubled; this is the result of population growth and changes in consumer habits. By 2020, consumption is estimated to remain at 16.2 and 21.5 kg (year per capita) $^{-1}$ for developing and developed countries, respectively (Chowdhury et al., 2010).
Untreated wastewater from the aquaculture industry has high loads of organic nutrients that include total organic carbon (TOC), ammonia nitrogen (NH^{3-} -N), nitrates (NO^{3-} -N) and phosphorus (PO_4-P). The discharge of this wastewater can generate an excess of nutrients, which leads to eutrophication in the receiving bodies of water. Therefore, the use of these effluents for the cultivation of microalgae can be interesting, not only for

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environmental sustainability through the biotreatment of the effluents but also for the economic sustainability of the cultivation stage (Trivedi et al., 2019).

The use of wastewater for the cultivation of microalgae allows the removal of elements present in the soluble fraction of the effluent, this being an alternative treatment and reuse of water, as well as the maximum use of the production of biomass and metabolites of commercial interest. (Christenson and Sims., 2011).

The first attempts for the commercial production of microalgae and cyanobacteria occurred with *Chlorella* and *Arthrospira* (*Spirulina*) sp, which were cultivated on a large scale in order to produce a nutritional supplement. At present, you can find multiple studies and advances that have allowed to generate products of industrial, cosmetic and pharmaceutical interest. For example, from the *Dunaliella salina* microalgae, β-carotene can be obtained, which is used as a pigment, provitamin A and antioxidant, likewise from *Scenedesmus spp.*, It is possible the production of lutein, used as an antioxidant and in ocular health, and *Chlorella spp.* algae, it is possible to obtain Canthaxanthin, a pigment used in the food industry, aquaculture, poultry and cosmetics (Borowitzka, 2018).

At a global level, it is possible to find clear and concrete examples of the use of wastewater to produce microalgae biomass to produce food and biofertilizers. Ansari et al (2017) and Guldhe et al (2017) evaluated on a laboratory scale the production of *Chlorella sorokiniana*, *Scenedesmus obliquus* and *Ankistrodesmus falcatus* in tilapia post-culture waters of South Africa using heterotrophic and mixotrophic strategies. Milhazes-Cunha and Otero (2017) demonstrated the feasibility of using algae cultures in conjunction with aerobic systems to recirculate water.

The present work shows the results of the evaluation of culture conditions necessary to produce biomass of *Scenedesmus obliquus*, *Chlorella vulgaris*, *Spirulina* sp and *Oscillatoria* sp in wastewater of pisciculture and determine the best conditions to produce carotenoids and phycocyanin.

2. Materials and methods

2.1 Fisheries wastewater

The wastewater was obtained from an intensive fish farming company in El Zulia (Norte de Santander, Colombia). For the cultivation of microalgae, the effluents were filtered twice with a cloth filter. The medium was UV-sterilized (4 Lamps of 15W, 5 minutes).

2.2 Algae strains

Chlorella vulgaris, Oscillatoria sp, and *Scenedesmus obliquus* were isolated from a local hotspring in Cucuta (Colombia) and stored at the Strain Collection of Universidad Francisco de Paula Santander (Colombia), *Spirulina maxima sp*. was purchased from NUTRE S.A.S. company. *Chlorella vulgaris* and *Scenedesmus obliquus* were maintained in modified Bold Basal medium, *Spirulina sp.* was maintained in Zarrouk medium (Zarrouk, 1966), and *Oscillatoria* sp in BG11 medium (Andersen *et al.,* 2005).

The four strains were cultured during 30 days in 2 L (1.5 L of working volume) GL45 clear glass bottles (4 for each strain) previously steam sterilized (120°C, 60 min). All the reactors were maintained under a light/dark cycle of 12h/12h and coupled to a bubbling aeration system mixed with CO_2 (1% v/v) with an air flow of 0.6 vvm.

2.3 Parameters of algae culture

The medium was UV-sterilized (4 Lamps of 15W, 5 minutes). In order to optimize the production of biomass and pigments, the wastewater was adjusted with the addition of nitrogen, phosphorus, and carbon (K_2HPO_4 + $NaNO_3$ + $NaHCO_3$), according to table 1 and table 2.

Table 1: Experiment design of modified culture medium for microalgae

Chlorella vulgaris			*Scenedesmus obliquus*		
Exp 1 (R1)	Fisheries wastewater $NaNO_3$ (25 g*L^{-1}) K_2HPO_4 (7.5 g*L^{-1}) KH_2PO_4 (17.5 g*L^{-1}) Na_2CO_3 (20 g*L^{-1})	Exp 2 (R2) Fisheries wastewater	Exp 3 (R3)	Fisheries wastewater $NaNO_3$ (25 g*L^{-1}) K_2HPO_4 (7.5 g*L^{-1}) KH_2PO_4 (17.5 g*L^{-1}) Na_2CO_3 (20 g*L^{-1})	Exp 4 (R4) Fisheries wastewater

Table 2: Experimental design of modified culture medium for cyanobacteria

Oscillatoria sp				*Spirulina maxima*			
Exp 5 (R5)	Fisheries wastewater $NaNO_3$ K_2HPO_4 (40 g*L^{-1}) Na_2CO_3 (20 g*L^{-1})	Exp 6 (R6)	Fisheries wastewater	Exp 7 (R7)	Fisheries wastewater $NaNO_3$ (2.5 g*L^{-1}) K_2HPO_4 (0.5 g*L^{-1}) $NaHCO_3$ (16.8 g*L^{-1})	Exp 8 (R8)	Fisheries wastewater + Zarrouk medium

2.4 Parameters for extraction

Was determined the possible effect of variables in the extraction of carotenoids and phycocyanin (pH, molarity, time and biomass/solvent ratio). Each of the experiments was performed using 20 mL of concentrated microalgae by centrifugation (3400 rpm, 15 minutes).

The extraction of the dyes was carried out according to the following method. The biomass was obtained by electroflotation (Castellanos-Estupiñan et al 2018) and re-suspended in 10 mL of phosphate buffer (K_2HPO_4, KH_2PO_4, 0.05M). One gram of glass beads (0.5 mm diameter) was added to the mixture. The solution was mixed using a vortex at maximum speed for 10 minutes. Once the process was finished, the sample was stored in a refrigerator at 4ºC for 24 hours. The extract was separated by centrifugation at 3400 rpm for 20 minutes.

Extracts from *Oscillatoria* sp and *S. maxima* (blue color) were collected and measured in a spectrophotometer at 620, 652, 562 and 280 nm. The calculation of the concentration of phycobiliproteins was done using the equations proposed by Bennett and Bogorad (1973) Eq(1-3):

$$C - PC(g/L) = \frac{OD_{620} - 0.474(OD_{652})}{5.34} \quad (1)$$

$$APC(g/L) = \frac{OD_{652} - 0.208(OD_{620})}{5.09} \quad (2)$$

$$PE(g/L) = \frac{(OD_{562} - 2.41(P-PC) - 0.849(APC))}{9.62} \quad (3)$$

Extracts from *C. vulgaris* and *S. obliquus* (green color) will be mixed with approximately 5 mL of cold chloroform and centrifuged at 3400 RPM for 8 minutes. The process will be repeated until the cells are colorless. The chloroform fraction will be removed and concentrated by rotoevaporation. The concentrated carotenoids will be resuspended in chloroform (approximately 3 mL) and read spectrophotometrically using the equation described by Přibyl et al. (2016) Eq(4):

$$Total\ carotenes\ (mg/L) = \frac{OD_{464} - 0.0222}{0.0325} \quad (4)$$

3. Results and discussions

Figure 1 shows the biomass production of the eight treatments with modification in the culture medium, these results show that the experiment R3 (6,5 g/L) stand out, which correspond to treatment with wastewater as a culture medium plus the addition of C, N, and P of the synthetic media. The use of fisheries wastewater as a culture medium has been used successfully for different microalgae; Blanco-Carvajal et al (2017, 2018) carried out studies where they determined that it is possible to obtain up to 2.0 g/L of biomass using *C. vulgaris*. On the other hand, Ansari et al., (2017) performed algae cultures in wastewater without the addition of additional nutrients, obtaining up several algae species, including *S. obliquus* (1,25 g/L), *C. sorikiniana* (1,51 g/L) and and *Ankistrodesmus falcatus* (2,25 g/L).

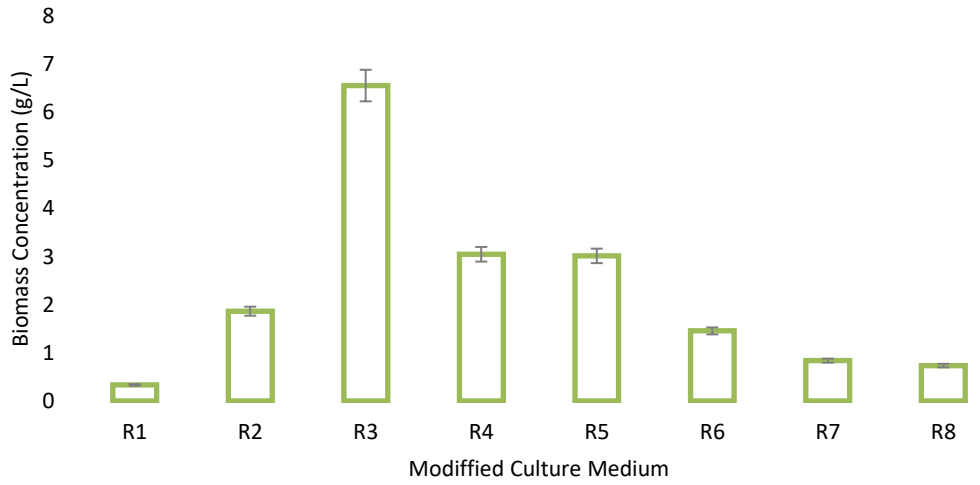

Figure 1: The concentration of biomass g/L of eight treatments with modified culture media from fishing wastewater

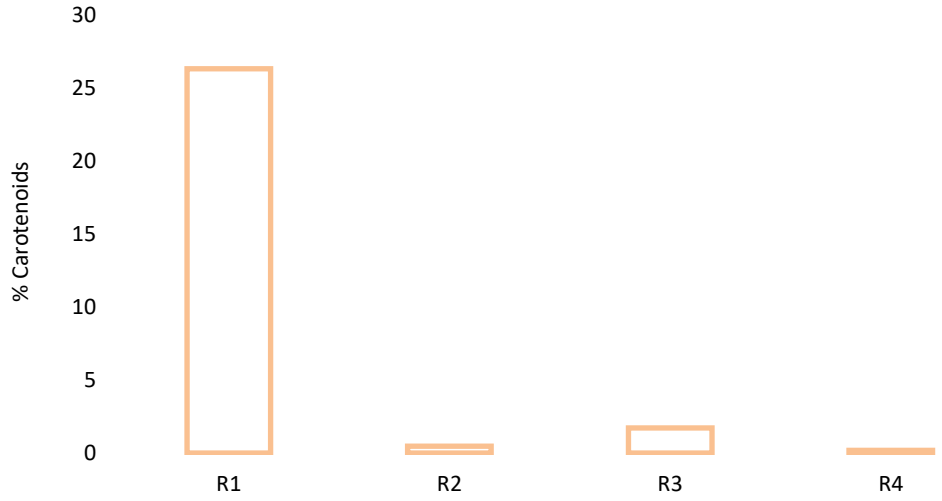

Figure 2: Percentage of carotenoids produced from chlorella sp and Scenedesmus cultivated in fishing wastewater modified

The percentage of carotenoids extracted from the microalgae is shown in Figure 2, and the results obtained from the R1 experiment stand out with 26% w/w, which makes it possible to highlight that the experiment R1 had the biomass production of 0,3 g/L (the lowest value of the present study).

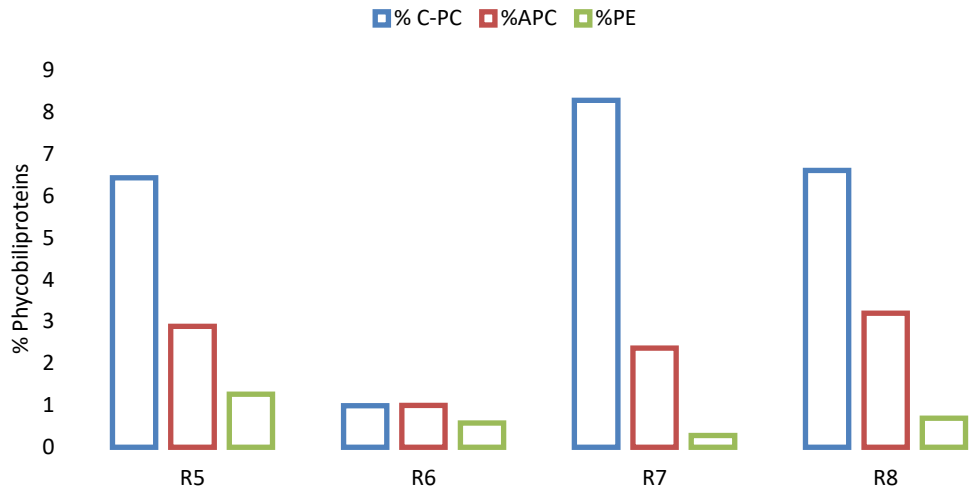

Figure 3: Percentage of Phycobiliproteins produced from Oscillatoria sp and S. maxima cultivated in fisheries wastewater modified

he production of microalgal biomass has three stages: cultivation (which involves inoculation and scaling), concentration, drying and in some cases extraction of metabolites of interest due to its high commercial value. Each of these stages involves a degree of complexity and costs, which in some cases may be restrictive. For the production of biomass as a source of animal feed (high protein and lipid content), the use of synthetic media can make the process unviable due to its high cost (Guldhe et al., 2017). This is due in principle to its low content of structured carbohydrates such as cellulose (Lam and Lee 2012); thanks to this, the contents of N and P are up to 3 times higher than in the higher plants (about 10% N and 1% P per unit dry weight) (Elser et al., 2000). This requires that microalgae cultures require higher concentrations of inorganic fertilizers than higher plants (Sialve et al., 2009); In addition, according to the Life Cycle Analysis (LCA) developed by Lardon et al. (2009), and Clarens et al., (2010) have found that the energy needed to obtain the nutrients necessary for the production of microalgae biomass is very high. Therefore, during the last years, the technical and economic feasibility of using sources with a high N and P content as domestic and industrial wastewater has been explored.

4. Conclusions

According to the results, the residual water enriched with K_2HPO_4, $NaNO_3$ and $NaHCO_3$ presented the best culture conditions for obtaining carotenoids (in *C. vulgaris* and *S. obliquus* with values of 2.6 and 1.7% p/p respectively) and Phycobiliproteins in *S. maxima* and *Oscillatoria sp* (10.9 and 11% p/p respectively). These results allow concluding that the residual water of fish systems is outlined as a suitable culture medium that can be used to produce metabolites of interest. Also, this culture medium must be enriched in order to increase the productivity of the system.

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