Bioconversion of post-culture wastewater from farm fisheries for the production of high-value algal biomass

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Abstract. Post-consumption water from inland fisheries possesses a tremendous environmental impact on aquatic ecosystems due to their high load of nutrients such as Nitrates, Phosphates, Urea and organic load. Due to the high cost of current water treatment systems, most of the waters from inland fisheries are discharged without any treatment, thus generating a significant environmental impact in rivers of different localities. Cyanobacteria are a group of photosynthetic microorganisms that can grow in different environments including wastewater. Among the most industrially exploited cyanobacteria, *Spirulina* (*Arthrospira*) *maxima* is the most relevant microorganism, due to its capacity to produce large quantities of protein and colourants (especially phycocyanins) for the food and feed industry. The objective of this project is to determine the maximum production capacity of *Spirulina* in post-consumption waters of fish farming as a system for the treatment of this type of water through the biological capture of the various nutrients and the production of biomass of industrial interest. *S. maxima* was produced on 3 different media (wastewater + Zarouk, wastewater + K2HPO4, NaNO3, NaHCO3 and wastewater without any modification) for 30 days. Results shown that *S. maxima* can effectively grow on wastewater supplemented with NaNO3 (2.5 g/L), NaHCO3 (16.8 g/L) and K2HPO4 (0.5 g/L) to obtain up to 1.18 g/L of total biomass and 0.23 g/L of phycocyanins. The scaling of culture at 10 L showed a minimal reduction on final biomass and phycocyanin (1.05 and 0.21 g/L respectively), this result indicates that the production of biomass and phycobiliproteins from *S. maxima* in wastewater from inland fisheries can be a possible candidate for the simplification of biomass and high-value metabolites process production.

1. Introduction
Wastewater from closed fish farming systems is an environmental problem due to high levels of nitrogen and dissolved inorganic phosphorus. The primary responsibility for these high contents is the food since according to [1], up to 75% of the food used is maintained in the form of nitrogen and phosphorus in the post-culture water. The latter contributes to the sustained increase in the concentration of organic waste and toxic compounds in aquatic systems [2-3].

During the last 50 years, significant efforts have been made to remove different nutrients from this wastewater, which prevent the eutrophication of water bodies close to the production systems and allow recirculating the treated water [1]. Currently there is a great diversity of biological and chemical methods that have been successfully used in the process of nutrient removal, such as: biological processes for the
elimination of nitrogen such as nitrification and denitrification [4], and chemical processes such as chemical precipitation for the elimination of phosphorus [5]. The last process, despite being useful, is a less environmentally friendly technique, since it entails the production of chemical residues and the formation of sludge as by-products, which are highly polluting agents for the environment [6].

The algae represent around 0.5% of the global biomass, however, they produce around 70% of the net oxygen on the earth, they also have a high growth rate, being between 10 to 50 times more efficient in CO₂ fixation than terrestrial plants [7], in addition to requiring less energy for the production of biomass [8]. The use of these microorganisms is considered as one of the technologies that contribute the most contributions to the conservation of the environment [3]; according to [9], the use of microalgae has the capacity to be economically and ecologically sustainable as long as its focus is under a multitrophic aquaculture (AMT) scheme. By the cultivation of microalgae or cyanobacteria in this type of systems, not only the removal of nutrients (such as nitrates and phosphates) can be guaranteed (by the high rate of biomass production), but the biomass produced can also be used in a sustainable manner [10] with economically viable products for the producer such as: concentrates for aquaculture, biofertilizers, and biofuels [6].

Currently, the use of microalgae and cyanobacteria for the removal of nutrients from inland fisheries wastewaters has only been tested on a laboratory scale and at a demonstration level in countries such as Spain [11], Denmark [12] and Belgium [13-14], South Africa [15-16] and China [6]. As far as the present proposal has been made, there are no studies at the demonstration or industrial level in Colombia, where the use of this type of microorganisms is evaluated. Taking into account the above, this Project aims to determine the production capacity of biomass and high-value industrial metabolites from spirulina maxima in ae growth in order to facilitate the recirculation of water and the use of wastewater from the national fish production.

2. Materials and methods

2.1. Microorganism

*Spirulina* (*Arthrospira*) *maxima* was acquired from NUTRÉ S.A.S (Colombia) and maintained in 0.5 L photobioreactors, with 0.3 L of Zarrouk media [17], a light intensity of 200 μmol m⁻² s⁻¹, photoperiod of 12:12 and 30 °C.

2.2. Post-culture water from inland fisheries

The wastewater was obtained from the production of *Oreochromis* sp in Manantial S.A.S (El Zulia, Norte de Santander, Colombia). The media was decanted to remove suspended solids and sterilised by exposure to UV (4 lamps. 5 min) according to the method described by [18]. After sterilisation, the water was stored in the pre-sterile flask until its use.

2.3. Experimental design

In order to determine the capacity of *S. maxima* to grow on the wastewater, the algae were initially adapted. Ten mL of 20 days old culture was inoculated in 250 mL of wastewater with extra nutrients (1/2 strength of Zarrouk media). The mixture was cultured during 30 days under a light intensity of 200 μmol m⁻² s⁻¹, photoperiod of 12:12 and temperature of 30 °C. Once the culture conditions were determined, scaling was carried out at a volume of 10 L in flat plate photobioreactors (15 cm x 35 cm x 50 cm). Conditions of growth are describe in Table 1.

| Reactor | 1 | 2 | 3 | 4 |
|---------|---|---|---|---|
| Conditions | Control (Zarrouk) | Wastewater | Wastewater + K₂HPO₄ | Wastewater |
|          | + Zarrouk | + NaNO₃ + NaHCO₃ | | |

Table 1. Conditions of growth.
2.4. Biomass quantification and analysis
The biomass concentration (in g/L) was obtained from dry weight [19]. Once every 5 days for 30 days (by triplicate) 5 mL of medium was removed and filtered using previously weighed 47 mm GF-C glass fibre filters (PALL Corporation). The filtered sample was dried overnight at 60°C and then stored in a desiccator until constant weight was obtained.

Total phycobiliproteins proteins were extracted by submerging the filtered biomass on 10 mL of phosphate buffer (0.15M, pH 7.0) with 1 g of glass beads (0.5 mm diameter). The mixture was disrupted with the assistance of a vortex at max speed for 15 minutes. At the end of the extraction time, the samples were stored in a refrigerator (4°C, 24 hours). After 24 hours in a refrigerator the sample was centrifuged (3500 rpm, 20 min). The supernatant (blue) was quantified by spectrophotometry at 620 nm, 652 nm, 562 nm and 280 nm. The calculation of the concentration of C-PC, APC and PE will be made using the Equation (1), Equation (2) and Equation (3) described by [20]:

\[ C \text{- PC (g/L)} = \frac{OD_{620} - 0.474(OD_{552})}{5.34} \]  
\[ APC (g/L) = \frac{OD_{552} - 0.208(OD_{620})}{5.09} \]  
\[ PE (g/L) = \frac{(OD_{562} - 2.41(P-PC) - 0.849(APC))}{9.62} \]

The purity of the extracts was determined using the Equation (4), Equation (5) and Equation (6) described by [21]:

\[ C \text{- PC} = \frac{OD_{620}}{OD_{280}} \]  
\[ APC (g/L) = \frac{OD_{552}}{OD_{280}} \]  
\[ PE (g/L) = \frac{OD_{562}}{OD_{280}} \]

3. Results and discussion

3.1. Biomass and phycobiliproteins production
Figure 1 shows the production of biomass for the different experiments after 30 days of culture. The highest concentration of biomass was obtained in the wastewater supplemented with Zarrouk media (1.78 g/L), followed by control (1.29 g/L) and the wastewater supplemented (1.18 g/L) with NaNO\(_3\) (2.5 g/L) + NaHCO\(_3\) (16.8 g/L) and K\(_2\)HPO\(_4\) (0.5 g/L). The cyanobacterium Spirulina sp is known for its capacity to grow in alkaline environments [22], therefore, by the addition of NaHCO\(_3\) the pH of the wastewater was modified close to 10, which allow the reproduction of the cyanobacteria.

The wastewater from fish farm is considered a suitable culture media since they had high levels of nitrogen compounds (ammonium, nitrites and nitrates), phosphates and dissolved organic carbon (COD) [3], which come from the high content of food without being consumed and the faeces of the individuals [1]. These high nutrient contents favour the growth of different microorganisms, including microalgae and cyanobacteria. Table 2 presents a summary of the most evaluated algae for the use of this type of effluent.

One of the most important features of the species of the genera Spirulina is his high protein and carbohydrates content, this characteristic makes this genus a candidate for the sustainable production of fertilizers as well as a good protein supplement in livestock feeds [27]. Phycobiliproteins (or PBP) is a group of colourants that have gained prominence in the food industries (especially sweets and soft drinks), cosmetics, nutraceuticals and pharmaceuticals, as well as in biomedical research and clinical
diagnostics [28]. To its non-toxic and non-carcinogenic attributes; therefore, it is considered a replacement for potentially toxic synthetic dyes [29]. According to the results (Figure 2) obtained for the concentration of phycobiliproteins (C-PC, APC and PE) there is no significant difference between the control and the wastewater supplemented with N, P and C.

![Figure 1. Biomass concentration for the different treatments.](image1)

**Table 2.** Comparison on biomass concentration.

| Strain                  | Biomass concentration (g/L) | Biomass productivities (mg/L*d⁻¹) | Industrial interest                                           | Reference |
|-------------------------|-----------------------------|----------------------------------|--------------------------------------------------------------|-----------|
| *Chlorella sorokiniana* | 3.490                       | 498.14                           | Biofuels and feed application                               | [16]      |
| *Chlorella vulgaris*    | 0.044                       | 7.30                             | Algal biomass production and nutrients removal               | [6]       |
| *Scenedesmus obliquus*  | 0.037                       | 6.20                             | Proteins, complex proteinogenic amino acids, and photosynthetic pigments | [23]      |
| *Desmodesmus armatus*   | 8.000                       | -                                |                                                              |           |
| (Chod.) Hegew           |                             |                                  |                                                              |           |
| *Chlorella sp. GD*      | -                           | 1296.00                          | Biodiesel production                                         | [15]      |
| *Ankistrodesmus falcatus*| 2.250                       | 160.79                           |                                                              |           |
| *Scenedesmus obliquus*  | 1.250                       | 89.61                            | Biofuels and feed applications                               | [24-26]  |
| *Chlorella sorokiniana* | 1.510                       | 107.86                           |                                                              |           |

![Figure 2. Ficocyanin concentration for the treatments.](image2)
3.2. Culture scaling

From the conditions obtained earlier, the culture volume (wastewater supplemented with N, P and C) was scaled up to 10 litres in flat-panel photobioreactors. According to the results presented in Figure 3 it is possible to demonstrate that *S. maxima* grow exponentially the first 10 days, followed by a stabilization until reach a final concentration of 1.05 g/L. This value is slightly lower to the one reported for the culture in 0.2 L (from 1.18 g/L to 1.05 g/L). This behaviour can be justified due to the change in the overall geometry of the photobioreactor employed for the process, since at 0.2 L the culture was maintained in a cylindrical bottle with a small light path (< 5 cm) and high turbulence regime; while on the rectangular PBR the turbulence was affected by its geometry and the light path was increased (15 cm); however the small reduction on the final concentration (less than 0.2 g/L) demonstrate the suitability of this cyanobacteria for the scaling of this kind of process.

Figure 3. Comparison on biomass concentration at 0.2 and 10 L.

Figure 4 shows that the total content of phycobiliproteins (C-PC, APC and PE) were not statistically different between the cultures in 0.2 an 10 liters. The synthesis and accumulation of these colourants varies from environmental factors such as light intensity, temperature and nutrient availability [30-31], however, the results obtained show that its final concentration is not affected by the increase in work volume or by the geometry of the reactor used. The preceding is promising for the production of this type of metabolites since according to [29], phycocyanin has been positioned worldwide as a natural food colouring whose price ranges between 25 and 1500 USD / kg.

Figure 4. Comparison on phycocyanin concentration at 0.2 and 10 L.
4. Conclusions
Wastewater from inland fisheries must be supplemented with NaNO₃ (2.5 g/L), NaHCO₃ (16.8 g/L) and K₂HPO₄ (0.5 g/L). NaNO₃ in order to obtain a fair biomass and total and total phycocyanin content (1.18 and 0.23 g/L respectively). The scaling of culture at 10 L showed a minimal reduction on final biomass and phycocyanin (1.05 g/L and 0.21 g/L respectively), this result indicates that the production of biomass and phycobiliproteins from S. maxima in cultures with wastewater from inland fisheries can be a possible candidate for the simplification of biomass and high-value metabolites process production.

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