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Production of *Chlorella vulgaris* Biomass on UV-treated Wastewater as an Alternative for Environmental Sustainability on High-Mountain Fisheries

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The sustained expansion of agricultural industry in Colombian high-mountain has led to an increased size of residues, especially untreated wastewater. This untreated water is an urgent matter for public and environmental health, not only by its nutrient concentration (composed especially of food residuals and feces) but also the presence of pathogens (virus, bacteria, etc.) which are discharged to the environment.
The overall objective of this work is to evaluate the effect of UV-treated wastewater from a high-mountain fishery as culture media for the production of *Chlorella vulgaris* as a sustainable method for nutrient and water recirculation on the fishery production system. The UV-canal efficiency was evaluated by the implementation of an experimental factorial design (time, distance of the UV-lamps towards the canal, number of UV-lamps and the sample concentration) using STATISTICA 7.0 software. Results shown that time (3 to 5 minutes) and the number of lamps (3-4) of 15 Watts eliminate completely coliforms from the samples.
After UV-treatment the resulting water was test as culture media for *C. vulgaris* production by the adjustment of C/N ratio (Sodium Carbonate/potassium nitrate) by the implementation of an experimental 2^3 factorial design. Results shown that higher nitrate concentrations (>0,22 g/L) and moderate carbonate concentrations (1 g/L) increase the final biomass concentration up to 4g/L in 20 days.

1. Introduction

According to FAO (2014), Limited Resource Aquaculture is one of the largest systems in Latin-America, where about 100,000 rural families have some kind of aquaculture system for the generation of proteins, bio fertilizers and others; This has allowed the improvement of food security in rural families in the region, transforming micro and small-scale aquaculture into an important income generator for small producers.
In Colombia, the fishing and aquaculture industry are two important sectors in the production of food for domestic consumption and exports, which contribute to overcoming poverty in rural areas (AUNAP-FAO, 2014), however, aquaculture has grown exponentially (13% annually during the last 27 years), displacing the fishing industry in 2011, due to the increase in production costs and the prolonged stagnation of the prices of final products. Colombian aquaculture is heavily concentrated in most of the departments of the Andean Region and in some departments of the Amazon and Orinoquía regions. These systems are characterized by being in agricultural families dedicated exclusively to aquaculture production; However, this accelerated

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growth entails certain restrictions among these can be highlighted three: food demand, high volume of fresh water used and high concentration of wastewater (Martins *et al.,* 2010; Mook *et al.,* 2012; Chen *et al.,* 2015).
The high volume of fresh water and wastewater are undoubtedly the main problem to solve. In these family systems, residual water is charged with high levels of nitrogen and phosphorus (Crab *et al.,* 2007), contributing to the increase in the concentration of organic residues and toxic compounds in aquatic systems (Vezzulli *et al.,* 2008; Gondwe *et al.,* 2012, Lananan *et al.,* 2014); which causes an imbalance in the ecosystem by favoring the reduction of available oxygen, thereby allowing the proliferation of harmful microorganisms and the disappearance of native aquatic species (Muthukumaran *et al.,* 2013;. Lan, 2014).
Options for the treatment of aquaculture wastewater have been sought over the years. Various biological and chemical methods have been successfully used to eliminate phosphate and nitrogen (Ebeling *et al.,* 2003), thus improving the quality of the effluent (Van rijn *et al.,* 1996). However, the chemical precipitation phosphorus is a less environmentally friendly technique, since it can generate sludge with high degree of contamination (Gao *et al.,* 2016). Other processes used are UV radiation, filtration and photosynthetic biological processes (Ludzack *et al.,* 1965). Bioremediation as an alternative to waste water treatment in the aquaculture industry is one of the most promising and environmentally friendly practices since it naturally involves bacteria and microalgae (Lananan *et al.,* 2014). This process has the function of transforming inorganic nutrients present in the medium (NO_3, PO_4, etc) and CO_2 into organic compounds such as proteins, carbohydrates, lipids and other macronutrients through photosynthesis (Gao *et al.,* 2016) which can be used as feed (Michels *et al.,* 2014).
Currently, microalgae culture is not yet a competitive method of nutrient removal in the intensive aquaculture industry, mainly due to the slow growth rate and the presence of competitive microorganisms (Gao et al., 2016). The present project focuses on the use of microalgae for the treatment of aquaculture wastewater effluents as a source of biomass for the Colombian aquaculture industry.

2. Materials and Methods

2.1 UV Disinfection

Prior to algae cultivation wastewater it was decided to eliminate the coliforms present through the use of UV lamps, however due to not knowing the operating conditions that allow the maximum elimination of this type of pathogens it was decided to use a 4^3 design of experiments (4 factors, 3 levels) in STATISTICA 7.0 (Statsoft, 2004) using as variables the number of lamps, concentration of medium to be treated, time and distance of exposure.
Because the software does not recognize qualitative values, a response range of 0 to 1 was assigned, being 1 the positive response (absence of coliforms) and 0 the negative response (presence of coliforms) (Table 1).
For the development of the experiments 15-Watt UV lamps were used in a radiation chamber with a 50 mL glass channel. After completion of each treatment, 2 mL of sample was inoculated aseptically into 65% (w/v) Fluorocult LMX medium (MERCK) and incubated for 24 hours at 37°C.

Table 1: Design of experiments for UV disinfection

Time (min)	Distance (cm)	# of UV lamps	WW concentration (%v/v)
1	2	2	50
3	4	3	75
5	6	4	100

2.2 Microalgae culture on UV treated wastewater

Chlorella vulgaris UTEX 1803 was purchased from the collection of strains from the University of Texas (Austin, Texas, USA). The algae were maintained in modified Bold Basal medium (Andersen *et al.,* 2005).
In order to determine the viability of microalgae biomass production, the concentration of carbon (sodium carbonate) and nitrogen source (Potassium nitrate) were adjusted using a 2^3 design of experiments (2 factors, 3 levels) (Table 2) without adding micronutrients.
Experiments were performed during 15 days in 500 mL GL 45 clear glass laboratory bottles previously steam sterilized (120°C, 60 min), each experiment was coupled to a bubbling aeration system with an air flow of 0.6 L/L of culture media. After 15 days, total biomass, total ash and total proteins were calculated according to Moheimani *et al*., (2013).

Table 2: Design of experiments for algae cultivation

KNO_3 (g/L)	Na_2CO_3 (g/L)
0,5	0,063
1	0,125
1,5	0,188

3. Results and Discussion

3.1 UV Disinfection efficiency

Results obtained from the experiments of UV show that the most influential variables in the elimination of coliforms were the exposure time (min) and the number of lamps used (Figure 1), where the optimal conditions are exposure times between 5-7 minutes and the use of 3-5 (15 Watt) UV lamps.
The use of UV treatments in agro-industrial and domestic sewage is extensive and varied, where the type of industry strongly influences the most relevant variables and operating conditions. According to the data reported by Velásquez *et al.,* (2016), a significant reduction of the total presence of coliforms (<565,000 Nmp/100 mL) in sewage from poultry farms requires the use of UV lamps of 20 Watts (360 nm) for 5 min; On the other hand, Hassem *et al*., (1999) reported that, in order to reduce the presence of coliforms up to 103 Nmp/100 mL in waste water from a domestic PTAR, only a UV lamp of 65 Watts (254 nm) and an exposure of 0,5 min is required.
In other studies, catalysts dissolved in water (homogeneous photocatalysis) have been used to generate hydroxyl radical's toxic to pathogens. According to Guimarães *et al*., (2010), coupling 100 Watt lamp with TiO_2 and an exposure time of 120 minutes can completely reduce (0%) the survival rate of different pathogenic microorganisms; On the other hand Watts et al (1995) applied a heterogeneous photocatalysis process (using TiO_2 coupled with 15 Watt lamp) in an effluent from a secondary treatment of domestic wastewater (Hannover, Germany), were able to reduce the number of total coliforms, ($5x10^5$ UFc/100 mL) and fecal coliforms ($1x10^4$ Ufc/100 mL). the differences in the efficiencies presented in the aforementioned works may be due to the wastewater source, which may affect the final concentration of coliforms and reduce (or increase) the final efficiency of the UV system.

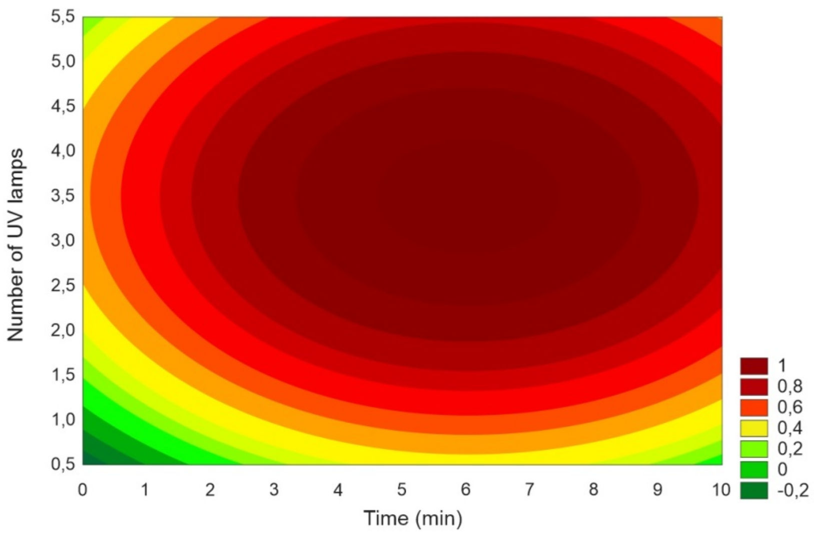

Figure 1. Surface response for coliform elimination

3.2 Biomass production on UV treated wastewater

After the UV-treatment results shown that in all the experiments the algae grown without any complication, and biomass was produced; however, due to the different levels on the nitrogen and carbon source some experiments were able to increase the concentration of biomass. The surface response (Figure 2) shows higher concentrations of nitrate (> 0.22 g/L) and concentrations of up to 1 g/L of carbonate, it is possible to obtain up to 4 g/L of biomass within 15 days. On the other hand, the results obtained for each of the experiments (Figure 3 left) from the modification in the concentration of sodium carbonate and potassium nitrate show that the highest concentration of biomass was obtained in the experiments with the highest

concentration of nitrogen (experiments 9 and 2) reaching values of up to 3.69 (g/L) for experiment 9 and 3.48 (g/L) for experiment 2.
The concentration of biomass obtained in the experiments is congruent with that described by Borowitzka (1999), since nitrogen is one of the nutrients that most affects cell growth; In addition, significant changes in the concentration of this nutrient in the medium can drastically modify biomass productivity, concentration and final composition especially pigments and proteins (Sánchez *et al.,* 2001). When checking the values for protein and ash for each of the experiments it was found that in each of the treatments the ash concentration in the biomass was close to 10% (w/w). On the other hand, the final protein concentration varies significantly from values as low as 40% (w/w) in the control to higher values (up to 60% w/w) in experiment 9.
These results are in accordance with those reported by Converti *et al.,* (2009), Uslu *et al*. (2011) and Ordog *et al.* (2012), where they emphasize that the final concentration of proteins depends directly on the presence of nitrogen in the medium; thus, at higher concentrations of assimilable nitrogen in the media it is possible to obtain higher protein concentrations in the final biomass.

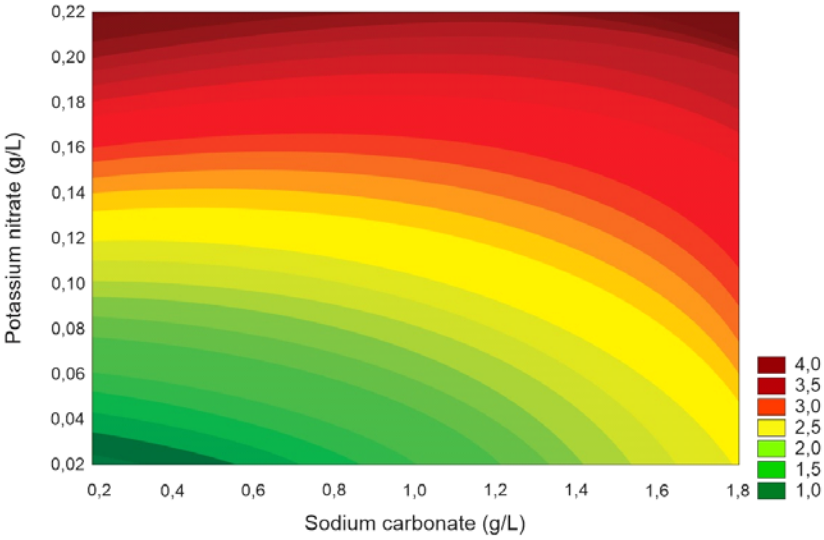

Figure 2. Surface response for biomass production

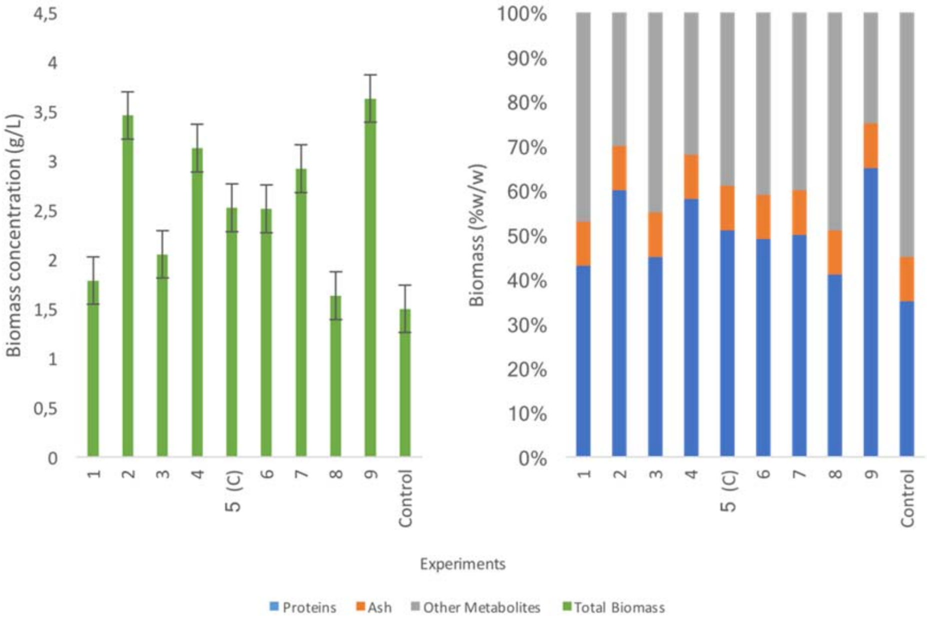

Figure 3. Biomass concentration (left) and total protein and ash in biomass (right).

4. Conclusions

The results obtained show that under the parameters studied, the number of UV lamps used, and the exposure time are the variables that most positively affect the removal of pathogens as coliforms in a 50 mL sample. Optimum conditions for removal are in the range of 3-5 15 Watt UV lamps for 5-7 minutes. Another important result to highlight is that this type of treatment does not negatively affect algae growth, therefore UV treatment can be a simple way for the elimination of microorganisms that can generate competition for algae.
On the other hand, biomass results show that aquaculture wastewater is rich enough in nutrients; therefore, apparently does not require to be supplemented with different micro and macronutrients (except nitrogen) for the production of microalgae. The latter poses a new scenario where the possibility of obtaining significant biomass concentrations (up to 4 g/L in 15 days) without adding extra nutrients (such as magnesium, potassium, sodium, etc.) that increase the cost of production opens of this type of systems.

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