@LIMENTECH CIENCIA Y TECNOLOGÍA ALIMENTARIA
ISSN Impreso 1692-7125/ ISSN Electrónico 2711-3035. Volume 21 No. 2, p. 138 - 152, year 2023
Facultad de Ingenierías y Arquitectura
Universidad de Pamplona

USE OF THE AIR-LIFT PHYTOREACTOR, FROM CHLORELLA VULGARIS MICROALGAE, FOR REMOVAL OF ORGANIC MATTER IN URBAN WASTE WATERS

UTILIZACIÓN DEL FITOERREACTOR AIR-LIFT, A PARTIR DE MICROALGAS CHLORELLA VULGARIS, PARA REMOCIÓN DE MATERIA ORGÁNICA EN AGUAS RESIDUOS URBANAS

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Received: August 13, 2023; Accepted: December 15, 2023

ABSTRACT

The environmental impact generated in natural water ecosystems by the discharge of wastewater produces eutrophication; which is the excessive growth of algae, due to the presence of high concentrations of salts. Therefore, in this investigation it was proposed to evaluate two strains of the Chlorella vulgaris microalgae (native and UTEX1803) and two concentrations of each (20 and 30%), for the removal of nutrients using urban wastewater from the municipality of Pamplona, North Santander. Initially, the physicochemical evaluation of urban wastewater was carried out. A concentric tube air-lift photobioreactor was designed, using the operating air flow a volumetric O_2 kLa mass transfer coefficient of 67.68h

-1, mixing time of 5.303 ± 0.0153 s and retention of gas in the reactor of 0.0219. In the start-up of the bioreactor for the removal of nutrients, the inoculums of the strains were determined in order to adapt them to the new substrate, by increasing cell growth. The 30% inoculum removal was 95% NO_3 and 83.3% PO_4 for the native strain and for the UTEX 1803 strain 92.5 and 91.2% for NO_3 and PO_4 respectively.

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Key words: Wastewater, microalgae, Chlorella vulgaris, air-lift photobioreactor

RESUMEN

El impacto ambiental generado en los ecosistemas de agua naturales por el vertimiento de aguas residuales produce la eutrofización; que es el crecimiento desmedido de algas, debido a la presencia de altas concentraciones de sales. Por lo anterior, en esta investigación se propuso evaluar dos cepas de la microalga Chlorella vulgaris (nativa y UTEX1803) y dos concentraciones de cada una (20 y 30%), para la remoción de nutrientes utilizando aguas residuales urbanas del municipio de Pamplona, Norte de Santander. Inicialmente, se realizó la evaluación fisicoquímica del agua residual urbana. Se diseñó un fotobiorreactor air-lift de tubos concéntricos, utilizando el flujo de aire de operación con un coeficiente volumétrico de transferencia de masa de O_2 kLa de 67,68h -1, tiempo de mezcla de 5,303 ± 0,0153 s y una retención de gas en el reactor de 0,0219. En la puesta en marcha del biorreactor para la remoción de nutrientes se determinó los inóculos de las cepas con el fin de adaptarlas al nuevo sustrato, mediante el incremento del crecimiento celular. La remoción con inóculos del 30% fue del 95% de NO_3 y 83,3% de PO4 para la cepa nativa y para la cepa UTEX 1803 del 92,5 y 91,2% para NO_3 y PO_4 respectivamente.

Palabras clave: Aguas residuales, microalga, Chlorella vulgaris, fotobiorreactor air-lift.

INTRODUCTION

Air-lift photobioreactors have been used in various applications, industrially in processes that require high mass transfer and good mixing, for example, for the aerobic production of microbial proteins, as well as in fermentation processes, cell cultures and as a wastewater treatment system.

The treatment of urban wastewater with microalgae has been studied since several years ago, in 1989 Pouliot et al. conducted laboratory scale experiments to determine the factors that influence the effectiveness of wastewater treatment with cyanobacteria, obtaining ammonia removal and phosphate removal of 95 and 62%, respectively.

In 1995 Craggs et al., evaluated the nutrient removal of two strains of marine microalgae denominated Phaeodactylum tricornutum in a primary wastewater effluent which they diluted in a 1:1 ratio with sterile seawater, obtaining removal results for strain B2 of 70% for ammonium and orthophosphate.

Martínez et al., in 2000, studied the elimination of nitrogen and phosphorus with the microalga Scenedesmus obliquus cultivated in urban wastewater which was previously subjected to secondary treatment, as well as studied the productivity of the biomass and its biochemical composition, the variables they manipulated in the cultures were agitation and temperature. They found a 100% reduction of ammonium, with a culture time of 188,33h, agitated and at 25°C.

In the same year Wong et al., studied the biosorption of nickel by two species of Chlorella, C. vulgaris (commercial) and C. maniata (local isolate) were cultured in nickel solutions with similar concentrations to the electroplating effluents.

Reyes et al., in 2012, evaluated the capacity of ammonium and orthophosphate removal by cultures of free microalgae and immobilized microalgae, using a mixed culture obtained from the wastewater from the UASB reactor of the Universidad Autónoma Metropolitana- Iztapalapa; and two cultures of Chlorella vulgaris and Spirulina subsalsa removal 50 and 74% for ammonium and orthophosphate, respectively.

The research project of Cartagena and Malo, in 2017 consisted of evaluating the microalgae Chlorella vulgaris in the removal of organic matter from wastewater from the wastewater treatment plant "El Salitre" located in Bogota - Colombia. They performed the adaptation of the microalgae to this substrate using culture volumes of 33 and 50%, then, they evaluated the growth of the microalgae already adapted using for each culture 10 and 30% of adapted microalgae.

For suspension cultures, the use of bioreactors with stirred tank and air-column systems is used. Likewise, bioreactors with pneumatic agitation have two principal types: air-lift bioreactors (air-lift) and bubble column bioreactors. Air-lift bioreactors have a number of advantages: it avoids bubble coalescence, distributes shear stresses throughout the reactor and increases the mass transfer rate (Wang y Zhong, 2001).

In 1998 Contreras et al., In 1998 Contreras et al. cultured the microalgae Phaeodactylum triconutum in a concentric tube air-lift reactor, the objective of this study was to determine the interaction.

The study by Sánchez et al., in 2016 characterized an internal circulation air-lift reactor and subsequently carried out the treatment of municipal wastewater where it decreased the organic load through pellets obtained by native fungi present in the green coffee bean.

MATERIALS Y METHODS

The purpose of the research work is the removal of nitrates and phosphates in urban wastewater from the municipality of Pamplona-Norte de Santander, through the cultivation system of the microalgae Chlorella vulgaris using an air-lift photobioreactor at laboratory scale. The project was developed from the following stages as shown in Figure 1:

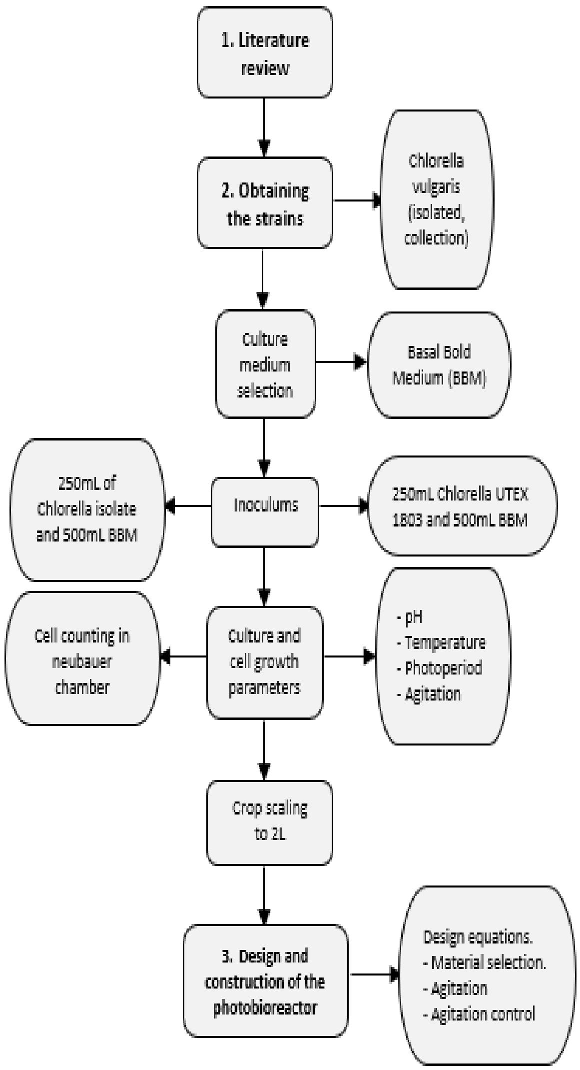

Figure 1. Diagram of the project process. Source: Author.

Initially, a bibliographic review of the state of the art of photobioreactors recommended for the cultivation of microalgae was carried out. Subsequently, it was determined the physicochemical characterization of the urban wastewater. The next stage consisted of obtaining the two strains of the microalgae Chlorella vulgaris, which were cultivated in Basal Bold medium (BBM) and finally the air-lift photobioreactor with concentric tubes was designed based on established equations.

RESULTS AND DISCUSSION

1. Obtaining the strains and culture variables

In this research we worked with two strains of Chlorella vulgaris, the first was a native strain obtained in the Biotechnology laboratory of the University of Pamplona in the Comagüeta Lagoon located in the municipality of Chitagá - Norte de Santander and the second strain, UTEX 1803, is a collection strain and was acquired by the GAIA laboratory of the University of Santander.

As culture medium for both strains, Basal Bold medium (BBM) was used. The cultures were scaled from a volume of 500mL to obtain 12 L of each one and for this an inoculum to medium ratio (BBM) of 1:2 was used. The culture conditions such as temperature and photoperiods were ambient; the average temperature of the municipality of Pamplona is 16°C and the light intensity was monitored with a lux meter during the 13 days of culture and this was at an average of 657,6153 ± 511,0391 lux. (see figures 2 and 3).

Figure 2. UTEX 1803 and native strains, respectively, grown at BBM. Source: Author.

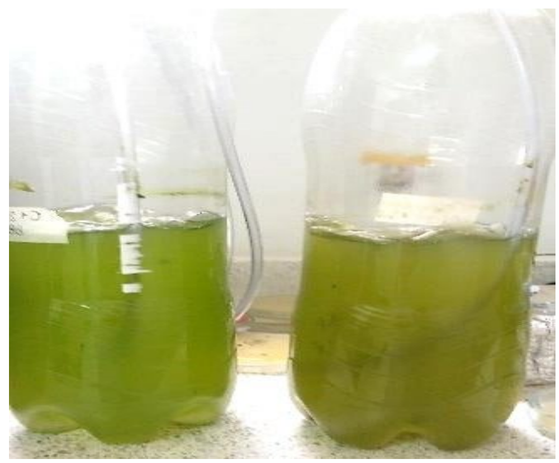

Figure 3. Native and UTEX 1803 strains, respectively, 13 days after inoculum 17. Source: Author

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On the other hand, agitation was carried out by means of fish tank compressors with an air flow of 2,5 L/min for cultures up to 2L, subsequently, when 12L of each strain (two gallons of 6L each one) were obtained, we worked with compressors of 5,5 L/min (See Figures 4).

Figure 4. Final scaling of the native strain, 12L. Source: Author.

2. Characterization of urban wastewater from the municipality of Pamplona and adaptation of the strains to the new substrate

For the characterization of the affluent, urban wastewater samples were taken from the municipality of Pamplona-Norte de Santander, the location of the discharge point and collection point of the affluent was at the transport terminal of the municipality. See Table 1.

Table 1. Physicochemical parameters of wastewater from the municipality of Pamplona-Norte de Santander

Parameter	Number of samples						
	1	2	3	4	5	6	Re 631 2015.
pH	7,31	7	8,03	7,52	7,91	7,75	6,29
Turbidity (NTU)	317	267	278	267	196	229	164
Color (PtCo)	1069	903	1231	1386	842	1123	434
OD (mg/L)	1,15	0,38	0,52	0,97	1,02	0,99	0,33
COD (mg/L)	833	857	1436	902	1103	894	433
DBO5 (mg/L)	524,7	539,9	904,6	568,2	694,89	563,2	272,7
Hardness (mg CaCO3/L)	40	130	300	150	250	260	40
Alkalinity (mg CaCO3/L)	210	125	265	245	255	235	75
Acidity (mg CaCO3/L)	90	180	65	75	70	80	60
Sulfates (mg/L)	73	52	25	48	44	78	117,92
Phosphates (mg/L)	20	23	15,7	15,4	17,7	16,4	25
Nitrates (mg/L)	5,5	17,3	7,3	6,3	5,6	5,2	7,3

Source: Author

By comparing the results obtained with the regulation of physicochemical parameters and the maximum allowable values for the discharge of domestic wastewater to surface water bodies (Resolution 0631 of 2015, the pH is within the established range, the chemical oxygen demand COD the maximum and minimum value are above the maximum allowable value, in total suspended solids TSS, the minimum value is in the range, however the maximum value and the average value are exceeded, likewise happened with settleable solids. On the other hand, the other parameters could not be compared since there is no limit in the regulation.

3. Design and construction of the air-lift photobioreactor

For the selection of the type of bioreactor that was to be designed and constructed, an extensive literature review was carried out, where it was found that air-lift bioreactors have several advantages over other bioreactors, advantages such as (Cortés *et al*., 2013):

- Increased mass transfer capacity due to its agitation system
- Highly effective fluidization of solids.
- Well defined flow patterns.
- Low shear stresses leading to cell growth as there is no stress or cell damage.
- Easy construction and low costs.

Bioreactors such as bubbler columns and air-lift bioreactors are widely used for the cultivation of microorganisms due to their pneumatic agitation, however, the main advantages of an air-lift bioreactor over bubbler columns are the improved mixing, in many cases the mass transfer coefficients are higher, because in air-lift bioreactors much higher gas velocities can occur. For example, in bubbling columns used in industry the surface velocity is in the order of 0,06 m/s and in air-lift bioreactors it is around 0,22 m/s resulting in an oxygen transfer coefficient of 0,143 s-1 (Chisti, 1989). In addition, this type of bioreactor is widely used as a culture system for wastewater treatment (Velasco, 2009; Arias, 2015; Sánchez *et al*.,2016).

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4. Specifications of the constructed reactor of the air-lift photobioreactor

In Table 2 the dimensions, ratios and total volume of the constructed photobioreactor are shown and in Figure 5 a schematic of the bioreactor is shown.

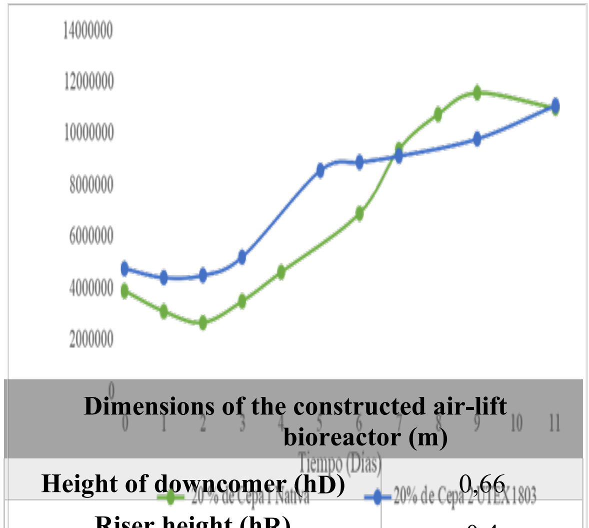
Table 2. Dimensions of the bioreactor

Dimensions of the constructed air-lift bioreactor (m)	
Height of downcomer (hD)	0,66
Riser height (hR)	0,4
Operation height (Lo)	0,54
Distance between diffuser and riser (L1)	0,05
Downcomer diameter (DD)	0,09
Riser diameter (DR)	0,058
Riser and downcomer cross-sectional areas	
AR (m^2)	0,0026
AD (m^2)	0,0064
Bioreactor relations	
hR/Lo	0,74
DR/DD	0,64
AD/AR	2,46
Total reactor volume (m^3)	0,0042
Volume of operation (m^3)	0,0034

Source: Author

In Figure 5, it can be observed that the concentric tube air-lift photobioreactor is completely built in 5mm thick acrylic, the gas inlet is at the bottom, therefore, the bioreactor has a diffuser plate that has 90 holes of 0,002m in diameter, for taking the sample or exit of the effluent with biomass, it was installed a tap of 1" at the bottom.

Figure 5. Concentric tube air-lift photobioreactor.



Source: Author

To determine all hydrodynamic parameters, water was used as the medium and the air flow rate was 6,41 L/min.

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5. Implementation of the constructed photobioreactor

In the reactor, the same inoculum concentrations of each microalgae were evaluated; the culture parameters measured during the treatments are shown below.

5.1 Inoculum of strains 1 and 2 at 20% in the constructed photobioreactor

In Figure 6, the cell growth and the parameters measured in the cultures in the photobioreactor are shown. The growth kinetics for the 20% inoculums of each strain in the reactor can be seen, in the growth of the native strain, a cellular decrease can be noticed between days 0 and 2, possibly the decrease occurred while the cells were adapting to the culture conditions since they were in a new system; on day 2 the exponential phase growth occurred, which lasted until day 9 of the treatment and, finally, the microalgae began its stationary phase, decreasing on day 11.

Figure 6. Growth kinetics of inoculums of strains 1 and 2 in the photobioreactor. Source: Author

In the growth kinetics of strain UTEX 1803, it can be noted that in the adaptation phase between days 0 and 2, the cell density decreased, but not in the same way as the native strain, which, on day 2 began its exponential growth phase until day 11, apparently this strain did not reach its stationary phase at the end of the treatment.

6. Evaluation of nitrate and phosphate removal in preliminary phase

To determine the decrease in nutrient concentration, initial and final samples were taken from each culture medium. The results of nutrient removal from the 20% cultures are shown in Table 3.

Table 3. Decrease in nitrate and phosphate concentrations with 20% inoculum of C. vulgaris.

CEPA	[ ] de NO_3 (mg/L)		[ ] de PO_4 (mg/L)	
	Initial	Final	Initial	Final
1	525,3	174,7	615,2	259,1
2	528,9	220,4	613,4	291,2

Source: Author

It can be seen that the microalgae Chlorella v. removed nitrates in greater proportion with a percentage of 66,74 and 58,32% for strain 1 and strain 2 respectively, similar results were reported in research conducted with the genus Chlorella (Valderrama *et al*., 2002; Andrade *et al*., 2006; Díaz y Ordoñez, 2006; Arias, 2015; Hernández y Labbé, 2014; Olarte y Valencia, 2016). On the other hand, the percentage of phosphate decrease for strain 1 and 2 was 57,88 and 52,52% respectively. The preliminary results of removal are satisfactory, the native microalgae has higher percentages of removal and this has a great advantage, since it encourages the isolation of these native microalgae and further research on these microorganisms; in addition to the use of biological resources from the environment.

In Table 4, it is shown the initial and final concentrations for the 30% cultures of the microalgae.

Table 4. Decrease in nitrate and phosphate concentrations with 30% inoculum of C. vulgaris.

CEPA	[ ] de NO_3 (mg/L)		[ ] de PO_4 (mg/L)	
	Initial	Final	Initial	Final
1	378,4	98,2	337,7	127,1
2	338,6	45,5	292	87,6

Source: Author

The percentages of nitrate removal for strain 1 and strain 2 were 74,04 and 86,56% and the percentages of phosphate removal were 62,36 and 70% for strain 1 and strain 2, respectively.

Comparing the results with the 20% inoculum, strain 1 increased the removal of nitrates and phosphates by 9,3 and 4,48% respectively; on the other hand, strain 2 increased the removal of nitrates and phosphates by 28,24 and 17,48%. A possible cause of the removal results of strain 1 could be the massive contamination of this culture, since there was cellular decrease in a very short time. In addition to this, another probable cause was that when taking the volume corresponding to 30% of strain 1 after homogenizing the culture, it was centrifuged, the culture was washed and with distilled water it was taken to the corresponding inoculum volume, the same was done with the inoculum of strain 2, however the cell concentrations in the inoculum of 20 and 30% for strain 1 had the same cell density, this can be seen in figures 16 and 18; contrary to what happened with the 20% and 30% culture of strain 2, since the cell concentration of the 30% inoculum culture of strain 2 had 602500 cells more than the 20% inoculum. Therefore, the acceptable treatment of the native strain can be explained.

7. Evaluation of urban wastewater performance before and after treatment

In Table 5 the estimation of physicochemical parameters after treatment of the culture is shown. For economic reasons, effluent characterization was only performed on one culture, so the treatment of native microalgae with 30% inoculum was selected, since this

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strain had the best performance in the treatments.

Table 5. Physicochemical parameters of wastewater, before and after treatment

Parameter	Initial	Final	% Decrease
pH	7,41	8,02	-
Turbidity (NTU)	251,42	3,74	98,51
Color (PtCo)	1031,25	168	83,71
OD (mg/L)	1,01	6,13	Not applicable
COD (mg/L)	947,75	445	53,05
DBO5 (mg/L)	597,08	369,35	38,14
Hardness (mg CaCO3/L)	202,92	80	60,57
Alkalinity (mg CaCO3/L)	203,33	30	85,25
Chlorides (mg Cl/L)	117,92	75	36,40
Acidity (mg CaCO3/L)	119,17	40	66,43

Source: Author

The increase in pH to an alkaline value is a consequence of photosynthetic activity in which the carbonate ions in the water supply the microorganisms with carbon in the form of CO_2, which favors the precipitation of orthophosphates and the elimination of ammoniacal nitrogen (Andrade *et al.*, 2006; Salazar, 2005).

On the other hand, the decrease in the other physicochemical parameters is evident, COD decreased with a percentage of 53,05%, a great decrease was observed in physical parameters such as turbidity and color with percentages of 98,51 and 83,71% respectively. Lazcano Carreño, 2016 in their research reported decrease in COD and DBO5 with 94,6% and 95,4% respectively. On the other hand, Cartagena y Malo, 2017, report a COD decrease of 81,8%,

Finally, an excellent adaptation of Chlorella vulgaris strains to urban wastewater could be observed, indicating that the treatment was satisfactory confirming the great advantages of the microalgae to easily adapt to contaminated media (Kumar, 2010; Luo *et al.*, 2011; Velasco *et al.*, 2011; Rodríguez et al., 2014; Arias, 2015; Cartagena y Malo, 2017).

CONCLUSIONS

The air-lift photobioreactor designed worked, optimizing the variables of the system, in this sense it improved: homogenization of the culture, cell growth, pH behavior, and dissolved oxygen; on the other hand, the geometry of the reactor allowed a good photosynthetic activity through the transfer of sunlight and there was no sedimentation and

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agglomeration of the microalgae in any of the treatments compared to the preliminary systems.

According to Resolution 0631 of 2015 (effluent discharge), the effluent to be treated was characterized by having high COD concentration, likewise, the values of volatile suspended solids and settleable solids exceeded the range of the standard. The treatment with the native strain obtained a decrease in COD, alkalinity and acidity of 53,05, 85,25 and 66,43% respectively.

The highest nitrate and orthophosphate removal were achieved in cultures with 30% inoculum of each strain. The native strain removed 95% nitrate and 83.8% phosphate, on the other hand, the UTEX 1803 strain removed nitrate and phosphate at92,5% and 91,2% respectively.

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