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Cultivation of *Chlorella* sp. for biodiesel production using two farming wastewaters in eastern Colombia

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Abstract: The production of biofuels using wastewater as a microalgae culture medium is a little explored technology, but with potential for success. In order to contribute to the knowledge of these technologies and their technical feasibility for microalgae growth, in this work the *Chlorella* sp. strain was cultivated in two types of effluents generated in an experimental farm located in eastern Colombia, before and after a biological treatment process. The consumption of the main nutrients that regulate growth and lipid production was evaluated, in order to extract, quantify, characterize and convert them into biodiesel. The results showed that *Chlorella* sp. growth and lipid production is more favourable in R2 medium of treated water than in R1 medium of raw water, mainly due to phosphorus limitation and higher N-NO_3 concentration in R2 compared to R1. In the R2 medium culture, a percentage of 42.54% of long-chain fatty acids was found, which is necessary to obtain a high quality biodiesel. Finally, the best transesterification experiment allowed reaching a fatty acid methyl esters (FAME) percentage of 90.1 ± 2.7%. In general, the results demonstrated the potential viability of using the wastewater generated in the San Pablo farm to produce biomass with lipid content to obtain biodiesel, finding that where the concentration of nutrients, mainly nitrogen, has a great influence on the microalgal metabolism for lipid accumulation.

Keywords: biofuel, *Chlorella*, lipids, microalgae, wastewater

INTRODUCTION

Biofuel production has taken great importance in recent years as one of the ways to mitigate the effects of climate change [Mohazzab *et al.* 2020] by replacing fossil energy based on crude oil, since the latter is composed of a complex mixture of hydrocarbons, kerosenes, resins, and in general, heavy molecules with high thermodynamic instability that generate problems during the production phases, affecting the level and quality of emissions generated to the environment [Alade *et al.* 2020]. This has led to the search for new technologies for their production through the use of microalgae, mainly because they represent an environmentally friendly solution able to reduce or eliminate the classical but polluting means of fuel production [Zikeli *et al.* 2020]. Numerous strains of marine or freshwater origin show a high lipid content to be transformed into biodiesel, including species of the genus *Chlorella* [Cuéllar-García *et al.* 2019; Ramírez-López *et al.* 2016], *Dunaliella* [Andreotti *et al.* 2019], and *Nannochloris* [Garzon-Sanabria *et al.* 2012; He *et al.* 2020]. On the other hand, in the last decade, the use of wastewater as a source of nutrients for cultivation has emerged as a promising and environmentally sustainable alternative [Caporgno *et al.* 2015; Chen *et al.* 2020; Rawat *et al.* 2013], taking into account the level of contamination and toxicity of some compounds present in these.

The production of biodiesel from microalgal biomass grown in wastewater has certain advantages over the use of plants, among them, extensive cultivation land is not needed because

they can grow in almost any enclosed space very quickly, they do not generate waste as occurs with palm oil production [Daochalermwong *et al.* 2020; Ferreira *et al.* 2020; Han *et al.* 2020]. It is a continuous, inexhaustible and non-polluting source of energy production because it does not mobilize fossil carbon but uses excess atmospheric carbon and with its consumption, compounds such as nitrates, phosphates and some heavy metals are reduced [Veronesi *et al.* 2015]. Therefore, this proposed combination increases the environmental benefit in CO_2 mitigation and preservation of valuable water resources, since, on many occasions, untreated wastewater rich in nitrogen and phosphorus is discharged into water bodies, leading to eutrophication of rivers and lakes and the appearance of harmful microalgae [Domańska *et al.* 2019; Gil-Izquierdo *et al.* 2021; Pełechata *et al.* 2016]. This study presents the design for the cultivation of the microalgae *Chlorella* sp. in two types of effluents obtained from an experimental farm located in eastern Colombia. The consumption of nitrogen and phosphorus present in these effluents, biomass production, extraction and quantification of lipids were evaluated. Finally, a transesterification process was carried out to evaluate the production of biodiesel.

MATERIALS AND METHODS

EFFLUENT SAMPLING

The wastewater samples used as culture medium in the biomass production stage and subsequent lipid production were obtained at the San Pablo Experimental Farm of the Francisco de Paula Santander University (Sp. Universidad Francisco de Paula Santander – UFPS), located in the municipality of Chinácota, Colombia; at the discharge points of domestic water and wastewater from washing stables, leaching of vermicompost and compost, and soil runoff (fertilizer residues) [Picos-Corrales *et al.* 2020]. Samples were collected and preserved by the refrigeration method in ice cellars [Torres *et al.* 2017] and transported for analysis in the Water and Environmental Biotechnology laboratories at UFPS.

The effluents were classified into R1 for raw farm wastewater and R2 for wastewater treated in an activated sludge pilot plant (Fig. 1) located at the Unit Operations Laboratory of the UFPS. This biological treatment system consists of three units, a 50 dm^3 aerated reactor with an air compressor and dissolved oxygen (DO), temperature and pH sensors; a 100 dm^3 storage tank with a diaphragm pump for continuous process operation; and a sedimentation unit with a turbidity sensor and a paddle as a mechanical agitator. The physicochemical characterization of effluents R1 and R2 was performed using the methods described in the "Standard methods for the examination of water and wastewater" [Rice *et al.* 2017], the parameters analysed were chemical oxygen demand (*COD*), NO_3, NO_2, PO_4, total suspended solids (*TSS*), volatile suspended solids (*VSS*) and pH.

STUDY ORGANISM AND GROWTH KINETICS AT 100 DM^3 SCALE

Chlorella sp. strain belonging to the INNOVALGAE Laboratory of the Francisco de Paula Santander University (Colombia) was used and maintained in basal BOLD medium [Cuéllar-García et al. 2019]. The inoculum was grown for 20 days in 10 dm^3 photobioreactors (7 dm^3 working volume) under a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 12 h / 12 h light/dark photoperiod and an airflow of 0.6 vvm. The bioprocess in 100 dm^3 photobioreactors was carried out under the following conditions: light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature of 28 ± 2°C, pH of 7 ± 1, photoperiod light/dark 12/12 and aeration of 1 vvm. The operating time was 10 days with periodic sampling every 24 h, where the variables of biomass production, nitrates, phosphates, COD, total lipids and lipid profile were monitored. Control cultures were used in all experiments.

Quantification of biomass. For the quantification of the produced biomass, aliquots of known volume were taken, vacuum filtered on nitrocellulose membranes of 0.22 µm pore diameter (Millipore ®) and dried at 80°C for 72 h. Previously, these membranes were brought to constant weight by keeping them at 80°C for 24 h [Jaimes -Duarte et al. 2012].

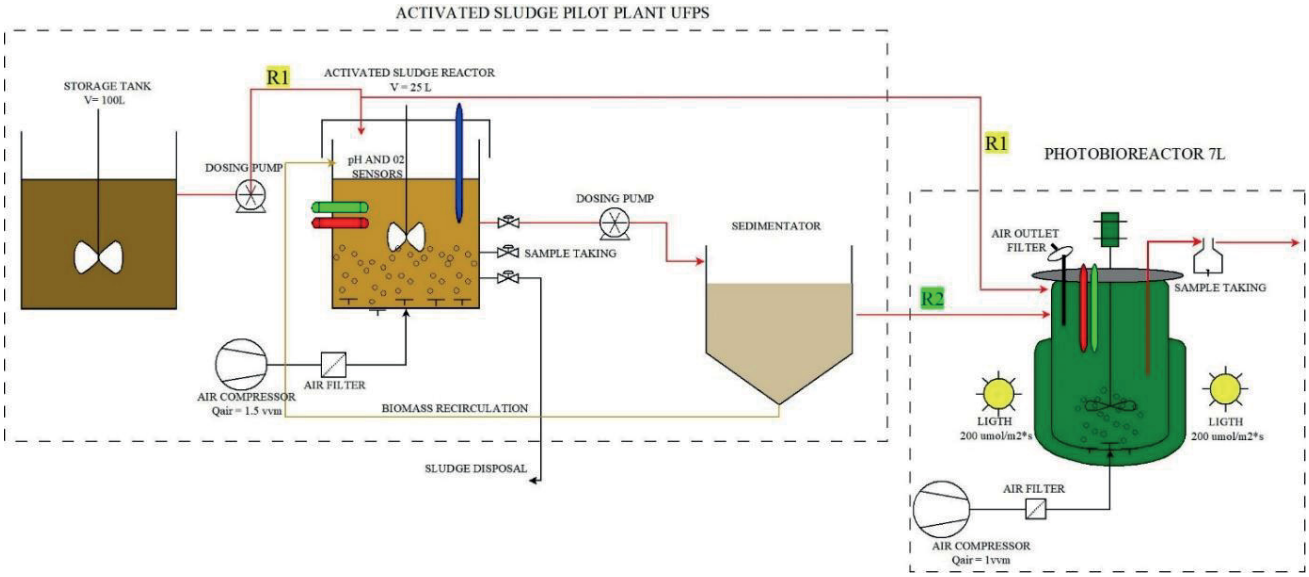


Fig. 1. Pilot plant of activated sludge – R1 and R2 treatment; source: own elaboration

Nitrogen quantification. Nitrate quantification was carried out using the Brucin-Sulfamic Acid method by absorbance [Jaimes-Duarte *et al.* 2012]. A 3 cm^3 sample was taken and diluted 1: 110 since initially the nitrate content exceeds the detection limit. Once all the reagents had been added, they were incubated in a water bath at 92°C for 30 min, and after half the time, the tubes were shaken to achieve a better homogenization of the mixture. Finally, they were allowed to cool, and the reading was taken in a spectrophotometer at a wavelength of 410 nm.

Quantification of phosphorus. The quantification of inorganic phosphorus was carried out by the modified method of Taussky and Shorr [Costa *et al.* 2009], which uses ammonium molybdate diluted 16% in sulfuric acid and ferrous sulphate. A 2 cm^3 sample was taken, the standard curve and the samples to be analysed were prepared. Once the colour reagents were added, it stands for 10 min, and then it was read at a wavelength of 660 nm.

EXTRACTION AND QUANTIFICATION OF LIPIDS

The biomass was dried in a Labcom freeze dryer and pulverized in a mortar. Subsequently and as part of the pretreatment, a cell disruption was performed with hydrochloric acid at a concentration of 0.6 M for 30 min to allow a greater extraction of lipids. Once this stage was finished, the extraction method reported by Ramírez-Fajardo *et al.* [2007], which consists of a Soxhlet system with a mixture of methanol-hexane as the solvent and an extraction time of 8 h. 1 cm^3 of methanol was added per gram of dry biomass, and it was left for 12 h at room temperature and stirring at 500 rpm. Afterward, it was centrifuged, and the solid phase was separated from the liquid phase, and then 0.2 cm^3 of hexane per cm^3 of used ethanol was added to the latter. Finally, the hexane phase was separated from the hydroalcoholic phase and the extracted oil was weighed.

TRANSESTERIFICATION SYSTEM

A low molecular weight alcohol (methanol) was used and mixed with a catalyst (sodium hydroxide) to form sodium methoxide ($Na+CH_3O^-$) in an exothermic reaction. This reagent was mixed with the oil, hydrolysing the triglyceride bonds to subsequently cause the splitting of the fatty acid into glycerine and ester chains and then react with the alcohol to give rise to biodiesel. In this regard, once the microalgae oil was obtained, it was kept in an oven for 1 h at 90°C to eliminate any water that might be present. Subsequently, in the pilot plant for obtaining biodiesel at UFPS (Photo 1), 15 g of the microalgae oil was added in the reactor of the pilot plant for obtaining biodiesel, which is equipped with a condenser and a mechanical agitator, and temperature control. The agitation was adjusted to 550 rpm and once the reaction temperature (60°C) was reached, the alcohol and catalyst were added in the proportions 5:1 and 10:1 with respect to the oil; the reaction time was 90 min. Once the reaction was finished, the mixture was left to stand in the plant separator funnel and neutralization was carried out to counteract the excess catalyst that did not react at a temperature of 60°C, then successive washes were carried out by adding 120 cm^3 of water purified the upper phase rich in esters by successive washes with distilled water. Finally, 12 was left in the decanter for

Photo 1. Pilot plant to obtain biodiesel UFPS (phot. *N.A. Urbina-Suarez*)

subsequent quantification of the fatty acid methyl esters (FAME's) by gas chromatography with mass selective detector (GC-MS).

ANALYSIS AND DATA PROCESSING

A factorial system was designed, the NSCC data analysis software was used to formulate the best medium for the growth of microalgae and the results were analysed and statistically verified using the Student's t-test of the SigmaPlot statistical package. Additionally, multifactorial analysis of variance (ANOVA) was applied to determine significant differences in the growth results in the related times.

RESULTS AND DISCUSSION

GROWTH KINETICS AT A 100 dm^3 SCALE

Several studies carried out on the increase in the productivity of microalgae show the existence of different aspects that can limit their growth, such as light and culture medium [Ji *et al.* 2016; Richmond 2004]. Table 1 shows the physicochemical characterization of effluents R1 and R2.

The objective of the characterization was to determine the concentrations of nitrate and phosphate, since these nutrients are the most important nutrients for microalgae cultivation

Table 1. Physicochemical characterization of effluents R1 (raw wastewater) and R2 (treated wastewater)

Parameter	Unit	Value in	
		R1	R2
Chemical oxygen demand (*COD*)	mg·dm^{-3}	2892.26 ± 31.39	523.2 ± 10.31
Nitrates		52,85 ± 2,04	167.52 ± 16.42
Nitrites		4.65 ± 0.32	7.05 ± 0.54
Phosphates		11.65 ± 0.61	4.21 ± 0.15
Total suspended solids (*TSS*)		1974.32 ± 42.66	635.38 ± 22.31
Volatile suspended solids (*VSS*)		1743.2 ± 21.2	483.27 ± 9.62
pH	–	7.4 ± 0.1	8.1 ± 0.1

Source: own study.

[Chu *et al.* 2019; Grobbelaar 2013]. These waters are generally composed of 95% water and the remaining suspended solids and dilution. The characterization evidences that in relation to *COD* the highest concentration is in the effluent R1, this is explained because it is raw wastewater without any treatment compared to R2 where the *COD* concentration is much lower, given that the wastewater has gone through an aerobic activated sludge process, where microorganisms have metabolized much of the organic substances. Phosphate concentrations were higher in R1 compared to R2, evidencing a removal of this compound by the activated sludge process. In relation to nitrates and nitrites in R1 the concentrations were lower compared to R2, this is explained that in an aerobic process, such as activated sludge, metabolic reactions occur that generate the oxidation of ammonia and ammonium salts and their subsequent conversion to nitrate [Bhattacharya, Mazumder 2021; Hernandez *et al.* 2018]. The results obtained regarding biomass production and nitrogen and phosphorus consumption are presented below.

Quantification of biomass. Figure 2 shows the production of *Chlorella* sp. in the medium R1 (raw wastewater) and R2 (treated wastewater). A higher concentration of biomass is observed in the R2 medium (1.76 ± 0.029 g·dm^{-3}) compared to the R1 medium (1.47 ± 0.036 g·dm^{-3}), possibly associated with the nitrogen level that limits the growth of the microalgae.

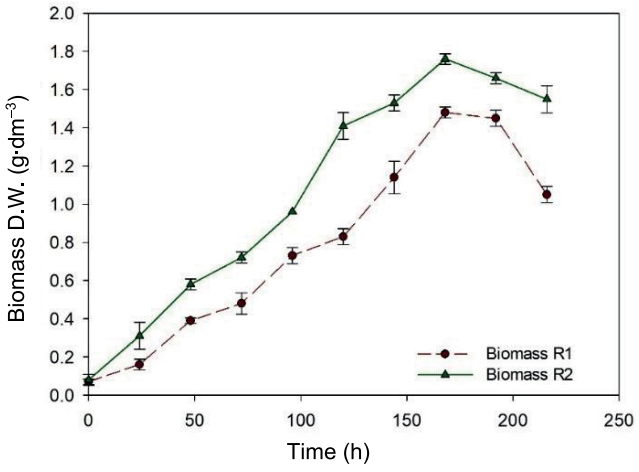


Fig. 2. Biomass production of *Chlorella* sp. (dry weight D.W.) in the 100 dm^3 photobioreactor in culture media R1 and R2; source: own study

Few studies have reported the growth of *Chlorella* sp. in wastewater obtained from experimental farms due to its high rumen content, and no studies have yet been reported using the effluent from an activated sludge plant as a growth medium for this microorganism. Authors who have used *Chlorella* sp. in domestic wastewater have reported biomass concentrations between 0.5 and 2.0 g·dm^{-3} [Jaimes-Duarte *et al.* 2012; Li *et al.* 2008; van Do *et al.* 2020]. However, in this study, wastewater with a much higher organic matter content than domestic wastewater was used, increasing its complexity to be assimilated by microorganisms. In the two cases analysed, *Chlorella* sp. was able to adapt to both raw and treated wastewater, reaching concentrations similar to those reported in the literature in heavy metal removal studies [Hassan *et al.* 2020; Perales-Vela *et al.* 2007].

Quantification of nitrogen and phosphorus. Figure 3 shows the consumption of nitrogen and phosphorus by the microalgae, where it is observed that there is a rapid consumption of both nutrients in the first 75 h, which represents about 75% of them. Subsequently, consumption is relatively low for both culture media. Some authors report that microalgae of the Chlorophyceae family have a high nutrient consumption in the first three to four days, and their growth does not stop, even when the concentration of nitrogen and phosphorus is relatively low in the medium [Hu *et al.* 2020; Leal Medina *et al.* 2017; Perales-Vela *et al.* 2007]. One of the reasons is that nitrogen and phosphorus are essential elements for the metabolism of microalgae, and these become part of some reserve products, such as lipids, carbohydrates, amino acids, and proteins [Cuéllar-García *et al.* 2019; Su 2021]. Comparing the results obtained in this work with those reported in the literature, it can be concluded that the consumption of nitrogen and phosphorus compounds present in the R1 and R2 media is similar and allowed adequate growth of *Chlorella* sp.

EXTRACTION AND QUANTIFICATION OF LIPIDS

Figure 4 shows the lipid content of the microalgae strain in the culture media R1 and R2 versus the maximum biomass concentration in dry weight.

The results show that the highest lipid content was obtained in the R2 medium with a value of 21.76 ± 1.06%, almost double that obtained with the R1 medium (12.08 ± 0.86%). Both experiments obtained higher values compared to the control that worked with the BOLD medium and where an average value of 9.84 ± 0.56% was reached. The explanation for this effect may be through nitrogen concentration, since it is considered the main regulator of growth and lipid accumulation. When a crop is exposed to adequate light intensity with nutrient limitation, the rate of cell division decreases; consequently, as a survival mechanism, the flow of carbon fixed by photosynthesis is diverted to lipid or carbohydrate synthesis [Florez *et al.* 2017; Ördög *et al.* 2012]. In this sense, when selecting culture conditions that favor lipid accumulation, it is necessary to maximize their volumetric productivity for each strain [Gao *et al.* 2019]. Therefore, the low nitrogen and phosphorus content of wastewater compared to synthetic media for microalgae growth explains the higher lipid generation in R1 and R2 media than in the control medium BOLD.

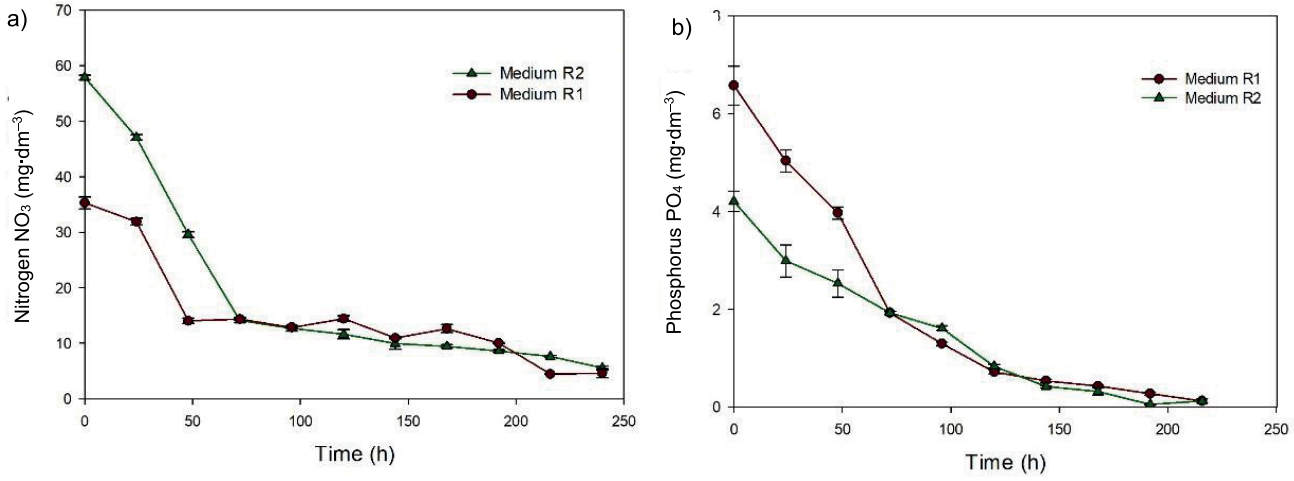


Fig. 3. Consumption of: a) nitrogen and b) phosphorus in the culture media R1 (raw wastewater) and R2 (treated wastewater); source: own study

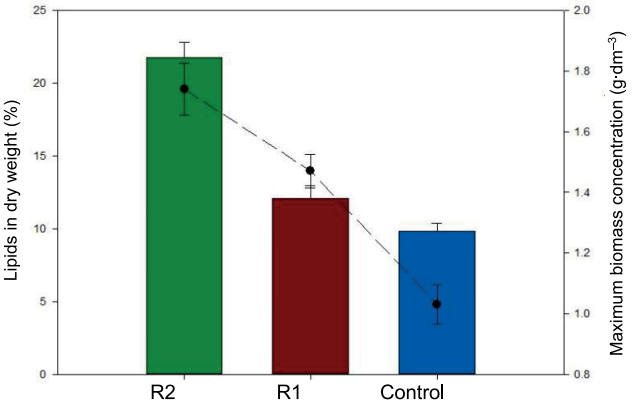


Fig. 4. Lipid content and maximum biomass concentration in culture media R1 (raw wastewater) and R2 (treated wastewater); the bars correspond to lipid content and the solid line corresponds to biomass; source: own study

The R2 medium presented a phosphorus limitation and a higher concentration of N-NO_3 compared to the R1 medium, which could explain that lipid production was higher in R2 than in R1. This phenomenon could occur because the level of complexity of the raw wastewater is higher than the treated effluent, therefore, the level of assimilation of nutrients seems to be better in the latter. Another aspect to consider is the competition for substrate, the level of bacteria and fungi is higher in raw wastewater, also competing for the same forms of nitrogen and phosphorus. When wastewater is treated, the resulting compounds are not as assimilable for most bacteria and fungi, but they are for photoautotrophic organisms such as microalgae. Table 2 presents the parameters and results obtained for the growth rate in the exponential phase of the culture. The productivity of both biomass and lipids was determined up to the maximum concentration reached.

Some authors have reported that the content of lipids in microalgae can vary from 1 to 90% of the dry weight, depending on the species and the cultivation conditions [Aziz *et al.* 2020; Chisti 2007; Luangpipat, Chisti 2016]. Subjecting the microalgae of the genus *Chlorella* sp. to stress conditions induces the synthesis and accumulation of large amounts of triglycerides, accompanied by considerable alterations in the composition of

Table 2. Kinetic parameters and lipid production of *Chlorella* sp. in culture media R1 (raw wastewater) and R2 (treated wastewater)

Parameter	Value in		
	control	R2	R1
Maximum biomass concentration (g·dm^{-3})	1.07 ± 0.02	1.74 ± 0.03	1.47 ± 0.04
Growth rate μ (d^{-1})	0.140 ± 0.001	0.245 ± 0.002	0.190 ± 0.0023
Lipids (% w/w)	9.84 ± 0.24	21.76 ± 0.67	12.08 ± 0.86
Lipid productivity (mg·dm^{-3} d^{-1})	15.00 ± 1.94	52.14 ± 3.24	25.42 ± 2.57
Biomass productivity (mg·dm^{-3} d^{-1})	143.85 ± 7.91	248.57 ± 12.73	210.00 ± 6.37
Lipid concentration (g·dm^{-3})	0.105 ±0.012	0.365 ± 0.026	0.178 ± 0.031

Source: own study.

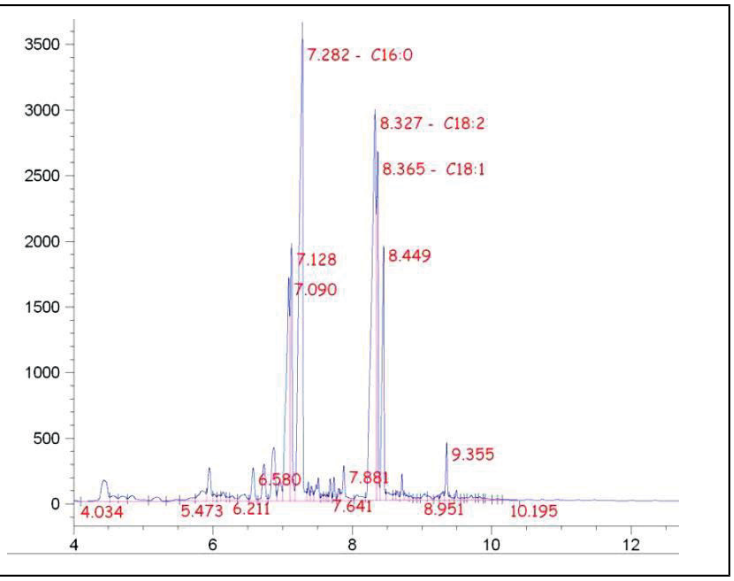
lipids and fatty acids. Similarly, it has been reported that under normal conditions without metabolic, nutritional, and environmental alterations, the lipid content of the microalgae of the Chlorophyta division can reach between 5 and 30% of the dry weight depending on the type of microalgae and the age of the culture [Garcia Gonzalez *et al.* 2020; Gouveia, Oliveira 2009; Saranya, Shanthakumar 2019; Spolaore *et al.* 2006].

Regarding the lipid productivity, values of 52.14 ± 3.24 and 25.42 ± 2.57 mg·dm^{-3}·d^{-1}were obtained for the R2 and R1 media, respectively. In the literature lipid productivities of 127.24 mg·dm^{-3}·d^{-1} are reported using *Chlorella vulgaris* in a culture with nutrient sufficiency and of 12.77 mg·dm^{-3}·d^{-1} under nutrient suppression conditions. Productivities achieved were relatively high compared to other studies and with the control medium (15.00 ± 1.94 mg·dm^{-3}·d^{-1}), which demonstrates the potential feasibility of the wastewater generated in the San Pablo farm as a medium for the production of biomass and biofuels.

For the transesterification process, the biomass that reached the highest percentage of lipids (culture medium R2) was chosen. This sample was processed and analysed by gas chromatography to determine the lipid profile found in Table 3.

Table 3. Lipid content of *Chlorella* sp. in culture medium R2

Fatty acid	% relative
Lauric acid C12:0	0.36
Myristic acid C14:0	0.39
Palmitic acid C16:0	12.40
Stearic acid C18:0	23.70
Oleic acid C18:1	14.42
Linoleic acid C18:2	25.10
γ-linolenic acid C18:3	9.50
Nonadecanoic acid C19:0	0.12
Arachidic acid C20:0	1.50
Eicosadenoic acid C20:2	0.23
Saturated	42.54
Unsaturated	57.30



Source: own study.

Obtaining high-quality biodiesel is based on long-chain fatty acids. Table 2 shows that the main fatty acids found in the microalgae grown in the R2 medium were: 25.10% of C18:2 (linoleic acid), 23.70% of C18:0 (stearic acid), 14.42% of C18:1 (oleic acid), 12.40% C16:0 (palmitic acid), 9.50% C18:3 (γ-linolenic acid).

The percentage of saturated fatty acids (SFA) and unsaturated (UFA) was 42.54 and 57.30%, respectively, which are like those reported in the literature for a culture of *Chlorella vulgaris* in synthetic medium BG-11 (37.18% SFA and 62.00% UFA). Other authors have reported SFA percentages for green microalgae between 20 and 50% [He *et al.* 2020; Ji *et al.* 2016]. Not all microalgal lipids are satisfactory for the production of biodiesel [Ramos *et al.* 2009]; however, the appropriate ones (fatty acids, free and covalently bound to glycerol and its derivatives) are frequently produced and constitute the largest fraction of total lipids, usually 20–40% [Chen *et al.* 2020; Chisti 2007; Sanghamitra *et al.* 2020].

TRANSESTERIFICATION SYSTEM

To carry out the transesterification process, the experimental model found in Table 4 was developed.

Table 5 presents the matrix of the eight experiments developed for alkaline transesterification, based on a 2^3 process. A total of 24 experiments were obtained in triplicate.

Table 4. Experimental design for lipid transesterification

Variable	Experiment	
	level 1 (–)	level 2 (+)
Alcohol-oil ratio	5:1	10:1
Amount of methanol	M-OH: 0.6% w/w	M-OH: 0.13% w/w
Reaction temperature	45°C ± 1	63°C ± 2

Source: own study.

Table 5. Variables from experiments performed for lipid transesterification

No.	Alcohol-oil ratio	NaOH concentration (% w/w)	Temperature (°C)
1	5:1	0.6	45
2	5:1	0.6	63
3	5:1	1.3	45
4	5:1	1.3	63
5	10:1	0.6	45
6	10:1	0.6	63
7	10:1	1.3	45
8	10:1	1.3	63

Source: own study.

In Figure 5 are observed the results obtained, where it is concluded that the best experiment was number 7 (90.1 ± 2.7%), which worked with a NaOH concentration of 1.3% w/w, a methanol molar ratio of 12:1, and a temperature of 45°C. The lowest percentage of FAME (fatty acid methyl esters) obtained was presented in experiment 2 (55.1 ± 2.7%) which worked with a NaOH concentration of 0.6% w/w, a methanol molar ratio of 6:1, and a temperature 63°C. In the same way, in this work, it was determined that the temperature and the concentration of NaOH considerably affect the obtaining of FAME, since the lowest percentages of FAME were obtained at high temperatures and low concentrations of NaOH. Some authors have reported that the best NaOH concentrations for obtaining FAME range between 0.6 and 1.5 % w/w, in the same way, they have reported that the increase in the molar ratio is directly proportional to obtaining FAME, despite ratios higher than 12:1 make it difficult to separate the glycerine [Ehimen *et al.* 2010; Nguyen *et al.* 2019; Plata *et al.* 2010] reported a percentage of FAME for synthetic oil from *Chlorella* sp. of 96.2% for a 12:1 molar ratio, a NaOH concentration of 1.5% w/w, and a temperature of 64°C.

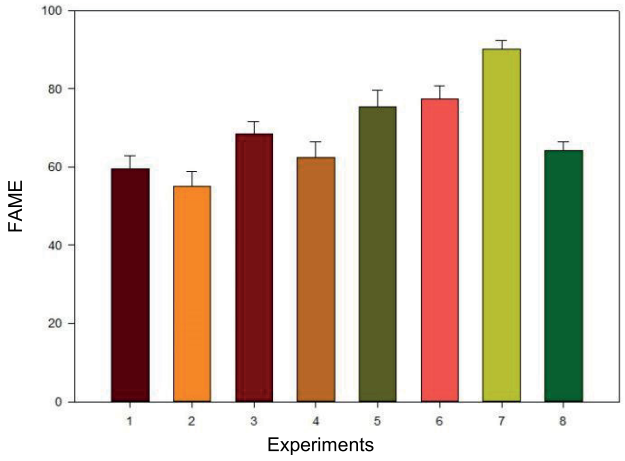


Fig. 5. Percentage of fatty acid methyl esters (FAME) obtained in the eight experiments performed; source: own study

CONCLUSIONS

In this study, the design for the cultivation of the *Chlorella* sp. strain in two types of effluents obtained from an experimental farm located in eastern Colombia was presented. The effluents were classified in R1 for raw wastewater from the farm and in R2 for wastewater treated in a pilot plant of activated sludge located. The results showed a higher concentration of biomass in the R2 medium (1.76 ± 0.029 g·dm^{-3}) compared to the R1 medium (1.47 ± 0.036 g·dm^{-3}), in addition to a consumption of 75% of nitrogen and phosphorus in the first 75 h. The R2 medium presented a phosphorus limitation and a higher concentration of N-NO_3 compared to the R1 medium, which could explain that the lipid production was higher in R2 (21.76 ± 1.06%,) than in R1 (12.08 ± 0.86%). This shows the importance of nutrients in microalgal metabolism since they become part of some reserve products, such as lipids, carbohydrates, amino acids, and proteins. Regarding the productivity of lipids, values of 52.14 ± 3.24 and 25.42 ± 2.57 mg·dm^{-3}·d^{-1} were obtained for the R2 and R1 media, respectively, which were relatively high compared to other studies and the control medium (15.00 ± 1.94 mg·dm^{-3}·d^{-1}). Furthermore, the main long-chain fatty acids found in the *Chlorella* sp. strain grown in R2 medium were C18:2 (linoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C16:0 (palmitic acid), and C18:3 (γ-linolenic acid), which are necessary to obtain high-quality biodiesel. Finally, the best transesterification experiment was number 7, which allowed reaching a percentage of fatty acids methyl esters of 90.1 ± 2.7%, working with a NaOH concentration of 1.3% w/w, a methanol molar ratio of 12:1 and a temperature 45°C. In general, the previous results demonstrate the potential feasibility of the wastewater generated in the San Pablo farm to produce biomass and biofuels.

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REFERENCES

Alade O.S., Amjed H., Mohamed M., Dhafer A., Abdulaziz A. 2020. Novel approach for improving the flow of waxy crude oil using thermochemical fluids: Experimental and simulation study. ACS Omega. No. 5 p. 4313–4321. DOI 10.1021/acsomega .9b04268.

Andreotti V., Solimeno A., Chindris A., Marazzi F., García G. 2019. Growth of *Tetraselmis suecica* and *Dunaliella tertiolecta* in aquaculture wastewater: Numerical simulation with the BIO_ALGAE Model. Water, Air & Soil Pollution. No. 230 p. 1–14. DOI 10.1007/s11270-019-4122-0.

Aziz M.M.A., Kassim K.A., Shokravi Z., Jakarni F.M., Liu H.Y., Zaini N., Tan L.S., Islam A.B.M.S., Shokravi H. 2020. Two-stage cultivation strategy for simultaneous increases in growth rate and lipid content of microalgae: A review. Renewable and Sustainable Energy Reviews. No. 119 p. 109621. DOI 10.1016/j.rser.2019 .109621.

Battacharya R., Mazumder D. 2021. Evaluation of nitrification kinetics for treating ammonium nitrogen enriched wastewater in moving bed hybrid bioreactor. Journal of Environmental Chemical Engineering. No. 9, 104589. DOI 10.1016/j.jece.2020 .104589.

Caporgno M.P., Taleb A., Olkiewicz M., Font J., Pruvost J., Legrand J., Bengoa C. 2015. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. Algal Research. No. 10 p. 232–239. DOI 10.1016/j.algal.2015.05.011.

Chen W., Liu Y., Song L., Sommerfeld M., Hu Q. 2020. Automated accelerated solvent extraction method for total lipid analysis of microalgae. Algal Research. No 51, 102080. DOI 10.1016/j.algal.2020.102080.

Chisti Y. 2007. Biodiesel from microalgae. Biotechnology Advances. No. 25 p. 294–306. DOI 10.1016/j.biotechadv .2007.02.001.

Chu F., Cheng J., Zhang Z., Ye Q., Zhou J. 2019. Enhancing lipid production in microalgae *Chlorella* PY-ZU1 with phosphorus excess and nitrogen starvation under 15% CO_2 in a continuous two-step cultivation process. Chemical Engineering Journal. No 375 p.121912. DOI 10.1016/j.cej.2019.121912.

Costa M., Lerchundi G., Villarroel F., Torres M., Schöbitz R. 2009. Producción de enzima fitasa de *Aspergillus ficuum* con residuos agroindustriales en fermentación sumergida y sobre sustrato sólido [Phytase production by *Aspergillus ficuum* in submerged and solid state fermentation using agroindustrial waste as support]. Revista Colombiana de Biotecnologia. No. 9 p. 73–83.

Cuéllar-García D.J., Rangel-Basto Y.A., Urbina-Suarez N.A., Barajas-Solano A. F., Muñoz-Peñaloza Y.A. 2019. Lipids production from *Scenedesmus obliquus* through carbon/nitrogen ratio optimization. Journal of Physics: Conference Series. Vol. 1388, 012043. DOI 10.1088/1742-6596/1388/1/012043.

Daochalermwong A., Chanka N., Songsrirote K., Dittanet P., Niamnuy C., Seubsai A. 2020. Removal of heavy metal ions using modified celluloses prepared from pineapple leaf fiber. ACS Omega. No. 5 p. 5285–5296. DOI 10.1021/acsomega.9b04326.

Domańska M., Boral A., Hamal K., Kuśnierz M., Łomotowski J., Płaza-Ożóg P. 2019. Efficiency of municipal wastewater treatment with membrane bioreactor. Journal of Water and Land Development. No. 41 p. 47–54. DOI 10.2478/jwld-2019-0026.

Ehimen E.A., Sun Z.F., Carrington C.G. 2010. Variables affecting the in situ transesterification of microalgae lipids. Fuel. No. 89 p. 677–684. DOI 10.1016/j.fuel.2009.10.011.

Ferreira Vela M.A., Acevedo-Páez J.C., Urbina-Suárez N., Rangel Basto Y.A., González-Delgado Á.D. 2020. Enzymatic transesterification ofwaste frying oil from local restaurants in east Colombia using

a combined lipase system. Applied Sciences (Switzerland). No. 10 p. 1–16. DOI 10.3390/app10103566.

Flórez-Miranda L., Cañizares-Villanueva R.O., Melchy-Antonio O., Martínez-Jerónimo F., Flores-Ortíz C.M. 2017. Two stage heterotrophy/photoinduction culture of *Scenedesmus incrassatulus*: potential for lutein production. Journal of Biotechnology. No. 262 p. 67–74. DOI 10.1016/j.jbiotec.2017.09.002.

Gao F., Yang H.-L., Li C., Peng Y.-Y., Lu, M.-M., Jin W.-H., Bao J.-J., Guo Y.-M. 2019. Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae *Chlorella* sp. Bioresource Technology. No. 282 p. 118–124. DOI 10.1016/j.biortech.2019.03.011.

Gonzalez E.G., De Carvalho J.C., Aulestia D.T.M., Gonzalez O.I.M., Soccol C.R. 2020. Bioprospection of green microalgae native to Paraná, Brazil using a multi-criteria analysis: Potential for the production of lipids, proteins, and carotenoids. Bioresource Technology Reports. No. 10 p. 100398. DOI 10.1016/j.biteb.2020.100398.

Garcia-Martinez J.B., Urbina-Suarez N.A., Zuorro A., Barajas-Solano A.F., Kafarov V. 2019. Fisheries wastewater as a sustainable media for the production of algae-based products. Chemical Engineering Transactions. No. 76 p.1339–1344. DOI 10.3303/CET1976224.

Garzon-Sanabria A.J., Davis R.T., Nikolov Z.L. 2012. Harvesting *Nannochloris oculata* by inorganic electrolyte flocculation: Effect of initial cell density, ionic strength, coagulant dosage, and media PH. Bioresource Technology. No. 118 p. 418–424. DOI 10.1016/j.biortech.2012.04.057.

Gil-Izquierdo A., Pedreño M.A., Montoro-García S., Tárraga-Martínez M., Iglesias P., Ferreres F., Barceló D., Núñez-Delicado E., Gabaldón J.A. 2021. A sustainable approach by using microalgae to minimize the eutrophication process of Mar Menor lagoon. Science of the Total Environment. No. 758, 143613. DOI 10.1016/j.scitotenv.2020.143613.

Gouveia L., Oliveira A. 2009. Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology & Biotecnology. No. 36 p. 269–274. DOI 10.1007/s10295-008-0495-6.

Grobbelaar J. 2013. Inorganic algal nutrition. Chapt. 8. In: Handbook of microalgal culture: Applied phycology and biotechnology. Ed. 2nd. Eds. A. Richmond, Q. Hu. Hoboken. Wiley-Blackwell p. 123–133.

Han J., Choi Y., Junghwan K. 2020. Development of the process model and optimal drying conditions of biomass power plants. ACS Omega. No. 5 p. 2811–218. DOI 10.1021/acsomega.9b03557.

Hassan R.M., Ibrahim S.M., Sayed S.A., Zaafarany I.A. 2020. Promising biocompatible, biodegradable, and inert polymers for purification of wastewater by simultaneous removal of carcinogenic Cr(VI) and present toxic heavy metal cations: Reduction of chromium (VI) by poly(ethylene glycol) in aqueous perchlorate solut. ACS Omega. No. 5 p. 4424–4432. DOI 10.1021/acsomega.9b03485.

He Y., Zhang B., Guo S., Guo Z., Chen B., Wang M. 2020. Sustainable biodiesel production from the green microalgae Nannochloropsis: Novel integrated processes from cultivation to enzyme-assisted extraction and ethanolysis of lipids. Energy Conversion and Management. No. 209 p. 112618. DOI 10.1016/j.enconman.2020.112618.

Hernandez-Martinez G.R., Ortiz-Alvarez D., Perez-Roa M., Urbina-Suarez N.A., Thalasso F. 2018. Multiparameter analysis of activated sludge inhibition by nickel, cadmium, and cobalt. Journal of Hazardous Materials. No. 351 p. 63–70. DOI 10.1016/j.jhazmat.2018.02.032.

Hu J., Liu H., Shukla P., Lin W., Luo J. 2020. Nitrogen and phosphorus removals by the agar-immobilized *Chlorella sacchrarophila* with long-term preservation at room temperature. Chemosphere. No. 251, p. 126406. DOI 10.1016/j.chemosphere.2020.126406.

Jaimes-Duarte D.-L., Soler-Mendoza W., Velasco-Mendoza J., Muñoz-Peñaloza Y., Urbina-Suárez N.-A. 2012. Characterization *Chlorophytas* microalgae with potential in the production of lipids for biofuels. CT&F - Ciencia, Tecnología y Futuro. No. 5 p. 93–102. DOI 10.29047/01225383.210

Ji M.-K., Yun H.-S., Hwang B.S., Kabra A.N., Jeon B.-H., Choi J. 2016. Mixotrophic cultivation of *Nephroselmis* sp. using industrial wastewater for enhanced microalgal biomass production. Ecological Engineering. No. 95 p. 527–533. DOI 10.1016/j.ecoleng.2016.06.017.

Leal Medina G.I., Abril Bonett J.E., Martínez Gélvez S.J., Muñoz Peñaloza Y.A., Peñaranda Lizarazo E.M., Urbina Suárez N.A. 2017. Producción de ácidos grasos poliinsaturados a partir de biomasa microalgal en un cultivo heterotrófico [Production of polyunsaturated fatty acids from microalgal biomass in a heterotrophic culture]. Revista Ion. No. 30 p. 91–103. DOI 10.18273/revion.v30n1-2017007.

Li Y., Horsman M., Wang B., Wu N., Lan C.Q. 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Applied Microbiology and Biotechnology. No. 81 p. 629–636. DOI 10.1007/s00253-008-1681-1.

Luangpipat T., Chisti Y. 2016. Biomass and oil production by *Chlorella vulgaris* and four other microalgae – Effects of salinity and other factors. Journal of Biotechnology. No. 257 p. 47–57. DOI 10.1016/j.jbiotec.2016.11.029.

Mohazzab B.F., Babak J., Nasrollahzadeh M., Mohaddeseh S., Varma R. 2020. Upgraded valorization of biowaste: Laser-assisted synthesis of Pd/ calcium lignosulfonate nanocomposite for hydrogen storage and environmental remediation. ACS Omega. No. 5 p. 5888–5599. DOI 10.1021/acsomega.9b04149.

Nguyen T.T., Uemura Y., Lam M.K., Mansor N., Lim J.W. 2019. Revealing the effect of reaction parameters towards alkyl group distribution in in-situ transesterification of *Chlorella vulgaris*. Energy Conversion and Management. No. 185 p. 223–231. DOI 10.1016/j.enconman.2019.01.113.

Ördög V., Stirk W.A., Balint P., Van Staden J., Lovasz C. 2012. Changes in lipid, protein and pigment concentrations in nitrogen-stressed *Chlorella minutissima* cultures. Journal of Applied Phycology. No. 24 p. 907–914. DOI 10.1007/s10811-011-9711-2.

Pełechata A., Pelechaty M., Pukacz A. 2016. Factors influencing cyanobacteria community structure in *Chara*-lakes. Ecological Indicators. No. 71 p. 477–490. DOI 10.1016/j.ecolind.2016.07.022.

Perales-Vela H.V, González-Moreno S., Montes-Horcasitas C., Cañizares-Villanueva R.O. 2007. Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae). Chemosphere. No. 67 p. 2274–2281. DOI 10.1016/j.chemosphere.2006.11.036.

Picos-Corrales L.A., Sarmiento-Sánchez J.., Ruelas-Leyva J.P., Crini G., Hermosillo-Ochoa E., Gutierrez-Montes J.A. 2020. Environment-friendly approach toward the treatment of raw agricultural wastewater and river water via flocculation using chitosan and bean straw flour as bioflocculants. ACS Omega. No. 5 p. 3943–3951. DOI 10.1021/acsomega.9b03419.

Plata V., Kafarov V., Moreno N. 2010. Optimization of third generation biofuels production: biodiesel from microalgae oil by homogeneous transesterification. Chemical Engineering Transactions. No. 21 p. 1201–1206. DOI 10.3303/CET1021201.

Ramírez-Fajardo A., Cerdán L., Robles-Medina A., Acién-Fernández F., González-Moreno P., Molina-Grima E. 2007. Lipid extraction

from the microalga *Phaeodactylum tricornutum*. European Journal of Lipid Science and Technology. No. 109 p. 120–127. DOI 10.1002/ejlt.200600216.

Ramírez-López C., Chairez I., Fernández-Linares L. 2016. A novel culture medium designed for the simultaneous enhancement of biomass and lipid production by *Chlorella vulgaris* UTEX 26. Bioresource Technology. No. 212 p. 207–216. DOI 10.1016/j.biortech.2016.04.051.

Ramos M., Fernández C., Casas A., Rodríguez L., Pérez A. 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technology. No. 100 p. 261–68. DOI 10.1016/j.biortech.2008.06.039.

Rawat I., Kumar R., Mutanda T., Bux F. 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Applied Energy. No. 103 p. 444–467. DOI 10.1016/j.apenergy.2012.10.004.

Rice E., Baird R., Eaton A. 2017. Standard methods for the examination of water and wastewater. 23rd ed. Washington, D.C. American Public Health Association. ISBN 9780875532875 pp. 1796.

Richmond A. 2004. Principles for attaining maximal microalgal productivity in photobioreactors: An overview. Asian Pacific Phycology in the 21st Century: Prospects and Challenges Developments in Hydrobiology. No. 173 p. 33–37.

Sanghamitra S., Deshmukh S., Narayan K.P. 2020. Effects of alternate nutrient medium on microalgae biomass and lipid production as a bioenergy source for fuel production. Materials Today: Proceedings. Vol. 28. Iss. P2 p. 659–664. 10.1016/j.matpr.2019.12.238.

Saranya D., Shanthakumar S. 2019. Green microalgae for combined sewage and tannery effluent treatment: Performance and lipid accumulation potential. Journal of Environmental Management. Journal of Environmental Management. No. 241 p. 167–178. DOI 10.1016/j.jenvman.2019.04.031.

Spolaore P., Joannis-Cassan C., Duran E., Isambert A. 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering. No. 101 p. 87–96. DOI 10.1263/jbb.101.87.

Su Y. 2021. Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. Science of The Total Environment. No. 762, 144590. DOI 10.1016/j.scitotenv.2020.144590.

Torres D., Sepúlveda S., Roa A., Gelvez J., Suárez N. 2017. Utilización de microalgas de la división Chlorophyta en el tratamiento biológico de drenajes ácidos de minas de carbón [Use of microalgae from the Chlorophyta division in the biological treatment of acidic coal mine drains]. Revista Colombiana de Biotecnologia. No. 19 p. 95–104. DOI 10.15446/rev.colomb.biote.v19n2.70429.

van Do T., Nguyen T., Tran D., Le T., Nguyen V. 2020. Semi-continuous removal of nutrients and biomass production from domestic wastewater in raceway reactors using *Chlorella variabilis* TH03-bacteria consortia. Environmental Technology and Innovation. No. 20, 101172. DOI 10.1016/j.eti.2020.101172.

Veronesi D., Ida A., D'imporzano G., Adani F.. 2015. Microalgae cultivation: Nutrient recovery from digestate for producing algae biomass. Chemical Engineering Transactions. No. 43 p. 1201–1206. DOI 10.3303/CET1543201.

Zikeli F., Vinciguerra V., Sennato S., Scarascia Mugnozza G., Romagnoli M. 2020. Preparation of lignin nanoparticles with entrapped essential oil as a bio-based biocide delivery system. ACS Omega. No. 5 p. 358–368. DOI 10.1021/acsomega.9b02793.