JOURNAL OF WATER AND LAND DEVELOPMENT
e-ISSN 2083-4535

Polish Academy of Sciences (PAN) Institute of Technology and Life Sciences – National Research Institute (ITP – PIB)

JOURNAL OF WATER AND LAND DEVELOPMENT
DOI: 10.24425/jwld.2022.140387
2022, No. 52 (I–III): 172–179

Prospects for using wastewater from a farm for algae cultivation: The case of Eastern Colombia

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RECEIVED 31.08.2020 REVIEWED 19.04.2021 ACCEPTED 24.05.2021

Abstract: In recent years, the technical and economic feasibility of using microalgae and cyanobacteria has been explored for the removal and exploitation of domestic, agricultural and industrial residual effluents with high C, N and P compounds content. To contribute to the understanding of the process and its technical viability for microalgae growth, the article discusses monitoring, flow determination, and physicochemical characteristics of two types of effluents generated in an experimental farm located in the east of Colombia, before (R1) and after biological treatment (R2). In general, the results showed the reduction of different parameters, such as total dissolved solids (*TDS*), hardness, salinity and phosphates after treatment with activated sludge. However, the conductivity value obtained in R1 and R2 showed the presence of a pollutant load. These findings can be attributed to the highest concentration of fats and oils in the water during early hours of the day. Finally, although the concentration of nitrates increased from 46.63 to 225.21 mg·dm^{-3} and phosphate decreased slightly from 9.65 to 6.21 mg·dm^{-3}, no inhibition was generated in the microalgae, as evidenced in the growth of the microalgal biomass in effluents after nitrate and phosphate removal above 80%.

Keywords: biological treatment, characterisation, microalgae, monitoring, nutrient wastewater

INTRODUCTION

Currently microalgae are emerging as the group of microorganisms with the greatest industrial potential due to the increasing applications of their different metabolites and derivatives to produce a variety of products. Microalgae are photosynthetic organisms that use solar energy, inorganic nutrients, and environmental CO_2 for their growth [Rawat *et al.* 2011]. Historically, the microalgae industry has developed around the production of pigments and proteins for human and animal consumption [Borowitzka 2015] by active exploitation of *Haematococcus pluvialis* to obtain astaxanthin, *Dunaliella salina* to obtain β-carotene, *Chlorella zofingiensis* to obtain lutein, and *Spirulina* (*Arthrospira*) *platensis* to obtain phycocyanin. These isolation technologies can reach the cost close to USD4000·kg^{-1} [Oilgae 2017]. For the production of both microalgae and cyanobacteria, three key elements are needed: 1) source of carbon (usually CO_2 or in organic form), 2) culture medium with sufficient concentration of nutrients, and 3) source of energy; in this case light known as photosynthetically active radiation [Chisti 2007; Lee 1999; Oncel 2013]. Among the most important nutrients for this group of microorganisms, nitrogen (in the form of NO_3), phosphorus (in the form of PO_4) and potassium (K) are the most important to carry out algal photosynthetic processes.

Various Life Cycle Analyses (LCA) of microalgae [Benemann *et al.* 2012; Clarens *et al.* 2010] indicate that the energy required to obtain necessary nutrients for the production of microalgae biomass is very high. To overcome these energy limitations, the technical and economic feasibility of using sources with a high content of N and P, such as industrial and domestic wastewater, has been explored in recent years. Although the use of domestic, agro-industrial, textile, and swine and aquaculture wastewater has been widely explored [Abdel-Raouf *et al.* 2012; Cai *et al.* 2013] for microalgae biomass production, the biomass is

used to obtain biofuels, biofertilisers, and animal feed, but there is no application for obtaining metabolites of high industrial value such as dyes or fatty acids. As an alternative to reduce the cost, a proposal has been made to produce algae using industrial effluents and by-products as a carbon source. This concept is known as biorefinery [Carvalho *et al.* 2018; Chen *et al.* 2018; Fazal *et al.* 2018; Gonzalez-Delgado, Kafarov 2011; Jaimes *et al.* 2012]. According to this principle, the potential of microalgae is used to remove nutrients, such as nitrogen and phosphorus, and incorporate microalgae into the biomass. This work presents the process of monitoring, flow determination and physicochemical characterisation of two types of effluents generated in an experimental farm located in eastern Colombia. The paper also evaluates the microalgal growth and removal of the pollutant organic load, nitrogen, and phosphorus compounds.

MATERIALS AND METHODS

SAMPLING

Wastewater samples were obtained in a composite way at the San Pablo Experimental Farm, located in the municipality of Chinácota, Colombia; at domestic water and wastewater discharge from barn washing, leaching of vermicompost and compost, and soil runoff (fertiliser residues) [Obeta *et al.* 2019]. Samples were collected from 8:00 a.m. to 2:00 p.m. in 30-minute intervals, preserved by refrigeration in cellars with ice, and quickly transported to the Water and General Biotechnology Laboratories of the University of Francisco de Paula Santander (UFPS). Monitoring was carried out weekly for four months.

QUANTIFICATION OF FLOWRATE

The measurement of the effluent flow was performed by the volumetric method using a 8 dm^3 container. The flow was monitored and measured from 8:00 a.m. to 2:00 p.m. every 30 minutes using Equation (1). At the end of the monitoring process, an average value was calculated based on flows measured during the day.

$$Q = \frac{V}{t} \tag{1}$$

where: Q = the flow ($dm^3 \cdot s^{-1}$), V = the volume collected (dm^3), t = the time (s).

PHYSICOCHEMICAL CHARACTERISATION OF EFFLUENTS

The physicochemical analysis is relevant for the handling and management of wastewater. It also provides information that helps to determine the nature and type of pollutants found in water [Bekkouch, Zanagui 2018]. Therefore, effluents to be used as a culture medium in the biomass production were classified into R1 for raw wastewater from the farm and R2 for wastewater treated in an activated sludge pilot plant located in the UFPS Unit Operations Laboratory. This biological treatment system (Fig. 1) consists of three units: a 50 dm^3 aerated reactor with an air compressor and dissolved oxygen (DO), and temperature and pH sensors; a 100 dm^3 storage tank with a diaphragm pump for continuous performance of the process; and a sedimentation unit with a turbidity sensor and a paddle as a mechanical agitator [Domańska *et al.* 2019].

Physicochemical analyses (Tab. 1) were performed to examine the physicochemical composition of the effluents R1 and R2, and to determine important compounds affecting the microalgae culture.

MICROORGANISM STUDIED

The *Chlorella* sp. strain belonging to the INNOVALGAE Laboratory of the Francisco de Paula Santander University (Colombia) was used and it was kept in a basal BOLD medium [Cuellar-García *et al.* 2019]. The strain was cultured for 25 days in 1 dm^3 photobioreactors (0.6 dm^3 working volume) under light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 12 h / 12 h light/dark photoperiod and an air flow of 0.6 vvm. All reactors were kept under a 12 h / 12 h light/dark cycle and coupled to a bubbling aeration system mixed with CO_2 (1% v/v) with an air flow of 0.6 vvm.

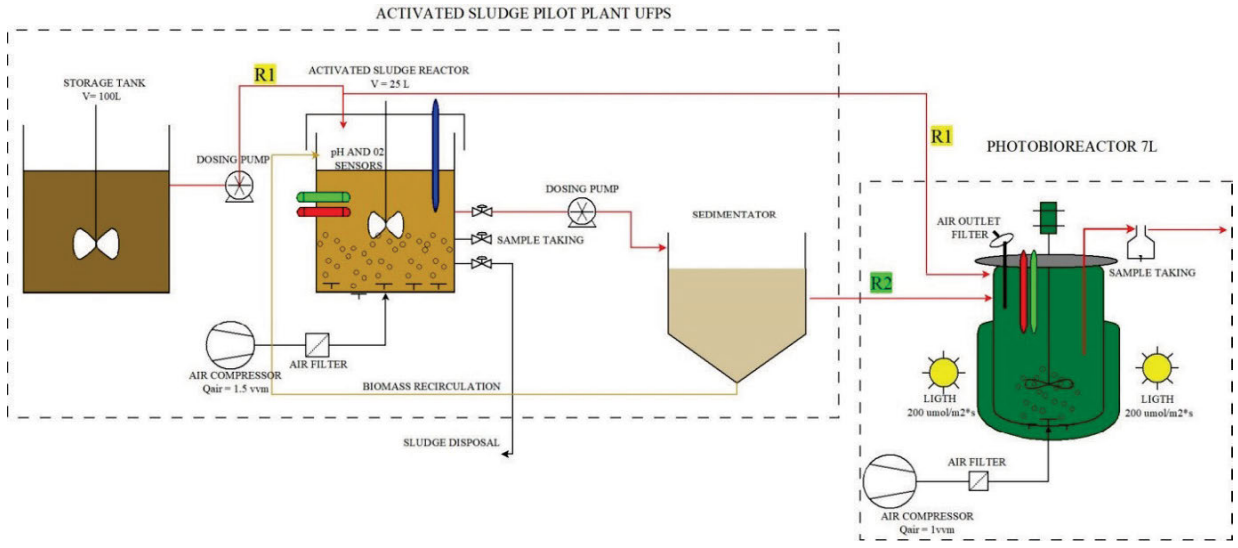

Fig. 1. Diagram of activated sludge pilot plant and photobioreactor of microalgae cultivation; source: own elaboration

Table 1. Physicochemical analysis performed on effluents before (R1) and after biological treatment (R2)

Analysis	Technique	Source
Chemical oxygen demand	colorimetric, reflux closed	APHA [2017]
Nitrates	Brucine method	
Total phosphorus	Taussky and Shorr method	Taussky and Shorr [1953]
Iron	colorimetric	APHA [2017]
Total dissolved solids	potentiometric	
pH	potentiometric	
Temperature	thermometer	
Dissolved oxygen	colorimetric (Winkler)	
Conductivity	potentiometric	

Source: own elaboration based on literature.

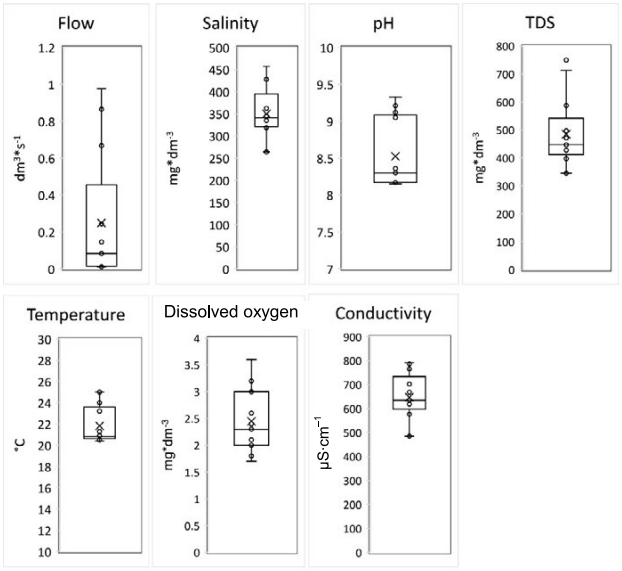

Fig. 2. *In situ* parameters of wastewater; *TDS* = total dissolved solids; source: own study

PHOTOBIOREACTOR AND CULTURE CONDITIONS

An airlift-type photobioreactor of 7 dm^3 (5 dm^3 working volume) was used, with a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 12 h / 12 h light/dark photoperiod and an air flow of 1 vvm. Effluents R1 (wastewater without treatment) and R2 (wastewater treated in activated sludge system) were used as the culture medium, whereas the BOLD basal medium was used as a control. A follow-up was carried out by taking samples every 48 h to evaluate biomass growth. The monitoring of *COD*, NO_3^-, PO_4^{3-} was performed at the beginning, middle, and end of the process. The biomass concentration was obtained from the dry weight [Jaimes *et al.* 2012; Moheimani *et al.* 2013]. Every 48 h a sample was taken to carry out the measurement (in triplicates). 10 cm^3 of medium was taken and filtered using 47 mm GF-C glass fiber filters (PALL Corporation). The filtered sample was dried overnight at 60°C and then stored in a desiccator until a constant weight was obtained. Phosphates were quantified using the colorimetric method with molybdovan-phosphoric acid. Nitrates were measured using the 4500-NO_3 ion selective method, and *COD* was quantified using the 522°C method as one of standard methods for the examination of water and wastewater 23rd edn. [Baird, Brigewater 2017].

RESULTS AND DISCUSSION

QUANTIFICATION OF FLOW RATE AND PHYSICOCHEMICAL PROPERTIES OF WASTEWATER

Figure 2 shows flow rate and average *in situ* physicochemical parameters measured during the four months of monitoring.

The results obtained in the quantification phase show that almost 80% of the wastewater from the Experimental Farm is generated during washing of different stables (between 8:00 and 9:30 a.m.). In these early hours of the day, the wastewater has the highest concentration of pollutant, i.e. fats and oils, which generates an increase in *COD*. In relation to dissolved oxygen, low levels of 1.7–3.2 $mg \cdot dm^{-3}$ are observed, which is common in this type of effluent due to the consumption of oxygen by the microorganisms present to degrade the organic matter contained in the water [Ortiz *et al.* 2017; Urbina-Suárez *et al.* 2006].

Figure 3 shows the average wastewater flow reached during the study period. Average values range between 0.18 and 0.30 $dm^3 \cdot s^{-1}$. Most of these effluents are generated by the excessive consumption of water for washing different stables in the livestock units. Hence, the times when this activity is carried out register high peaks in the flow of wastewater. Although there is a variation of the average flow from month to month, the results of *TDS*, salinity, and average conductivity are similar.

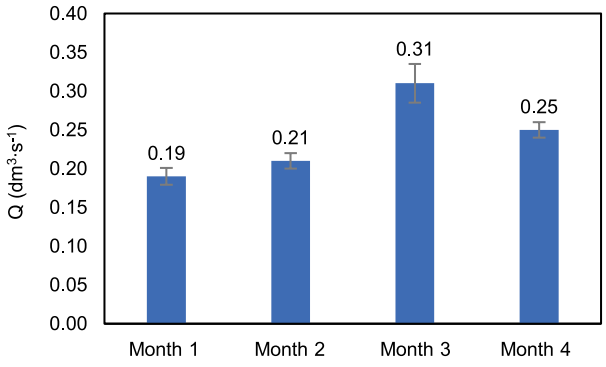

Fig. 3. Average flow rate (*Q*) per month; source: own study

PHYSICOCHEMICAL CHARACTERISATION OF EFFLUENTS R1 AND R2

Figure 4 shows the average results for physicochemical parameters of R1 and R2 effluents. It is observed that *COD* in R1 is considerably higher compared to R2, because in the latter effluent, there are aerobic degradation reactions that reduce the concentration of organic components by the microorganisms present in the activated sludge. [Cramer *et al.* 2019; Ortiz *et al.* 2017; Romero Rojas 1998].

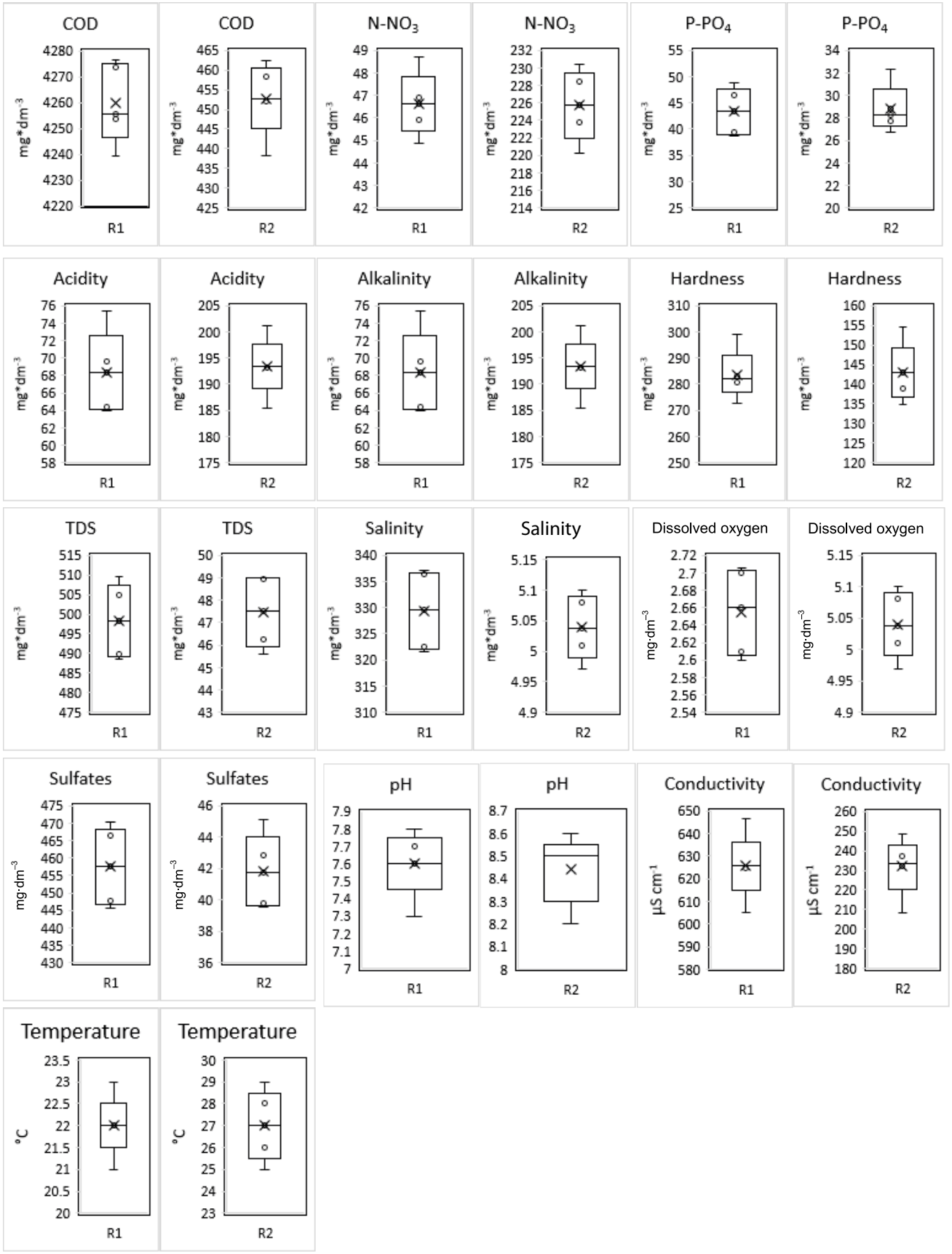

Fig. 4. Physicochemical parameters of raw wastewater (R1) and treated wastewater (R2); *COD* = chemical oxygen demand, *TDS* = total dissolved solids; source: own study

The concentration of nitrates and phosphates were measured because they are fundamental elements in autotrophic growth in the production of microalgae biomass [Cai *et al.* 2013; Jaimes *et al.* 2012; Torres *et al.* 2017]. Generally, wastewater of similar types to those generated in the farm consists of 99% water and 1% suspended solids and solutions. Solids may be classified into inorganic and organic; the former are mainly made up of nitrogen, phosphorus, chlorides, sulphates, carbonates, bicarbonates, and some toxic substances, such as arsenic, cyanide, cadmium, chromium, copper, mercury, lead, and zinc [Hernandez-Martines *et al.* 2018; Ouali *et al.* 2018]; while the latter can be nitrogenous (proteins, urea, amines, and amino acids) and non-nitrogenous (cellulose, fats, and soaps) [Urbina-Suárez *et al.* 2006].

Protein synthesis normally depends on an adequate supply of nitrogen [Lam, Lee 2012; Sze 1998]. An increase in the availability of inorganic nitrogen leads to an increase in the abundance of primary producers. However, high levels of inorganic nitrogen that cannot be assimilated by ecological systems can cause adverse effects on less tolerant organisms [Camargo, Alonso 2006]. In the case of microalgae cultures, most of these microorganisms can use NO_3^-, NO_2 or NH_4^+ as a source of nitrogen. However, the use of inorganic forms involves the first step of uptake and subsequent processing to integrate into the organic matrix of the cell. For algae, it is more favourable to use ammonium when it is available in its free form as they accept it into metabolic pathways directly; this is not the case for nitrate which must first be transformed into nitrite and then into ammonium through four energy-requiring reduction steps [Cuellar-García *et al.* 2019; Sze 1998]. Therefore, when ammonium and nitrate appear in sufficient quantities, most algae use ammonium first and then nitrate, once ammonium ions are depleted. This preference is related to the control of nitrate assimilation that causes feedback, inhibition, and repression of enzymes responsible for nitrate reduction [Richmond 2004; Yang *et al.* 2000]. The results show (Fig. 4) that the concentration of nitrogen measured in the form of nitrate is higher in R2 than in R1 due to the type of treatment used for the raw wastewater and the level of bacteria concentration in it. On the one hand, when working with an aerobic activated sludge system, a nitrification process takes place, which causes the oxidation of ammonia and ammonium salts, and their subsequent conversion to nitrate. On the other hand, the diversity of microorganisms in raw wastewater generates competition in nitrogen assimilation, where the availability of nitrogen for microalgae is limited because the bacteria growth rate is higher than that of microalgae [Rosa *et al.* 2010; Torres *et al.* 2017].

As for the action of phosphorus, it plays an important role in most cellular processes, especially those related to the generation and transformation of metabolic energy, essential for the growth and reproduction of microalgae [Cuellar-García *et al.* 2019; Yang *et al.* 2000]. Total phosphorus present in the aquatic environment comprises dissolved inorganic phosphorus, organic phosphorus compounds, and dissolved organic phosphorus in the form of suspended particles [Sze 1998]. Soluble phosphate is present in small amounts in natural waters, even at lower concentrations than nitrogen [Marselina, Burhanudin 2018], except in some waters contaminated by certain organic materials. While algae can utilise nitrogen in different ways, phosphorus must be assimilated almost exclusively in the form of phosphates, and it is the concentration of phosphorus that determines the algal growth rate. The maximum algal biomass that can support the total amount of phosphorus present depends on the number of orthophosphates remaining available to growing cells [Li *et al.* 2014; Sze 1998].

The results obtained (Fig. 4) show that the phosphate concentration is slightly lower in R2 compared to R1, mainly due to the consumption by microorganisms present in the activated sludge; however, the concentration that was present in the effluent does not inhibit the microalgae, as shown in Figure 5, where the growth of the microalgal biomass in this effluent was evidenced.

In general, acidity (28.76 mg·dm^{-3}), hardness (142.85 mg·dm^{-3}), *TDS* (47.89 mg·dm^{-3}), salinity (189.33 mg·dm^{-3}), sulphates (41.98 mg·dm^{-3}) and conductivity (Fig. 4) in R2 are lower than those found in R1 due to the effects of the treatment with activated sludge. The conductivity value in R1 (625.34 ±21.93 µS·cm^{-1}) shows excess contamination with mineral nutrients [Burzyńska 2019] and pH shows that both types of effluents are slightly neutral with a tendency to alkaline (between 7.6 and 8.4). Temperature plays an important role in the solubility of salts and is influenced by the origin of water [Merzougui *et al.* 2019]. Finally, by reducing the pollutant organic load, the availability of DO in R2 is higher.

BIOMASS PRODUCTION AND REMOVAL OF *COD*, NITRATES AND PHOSPHATES

Figure 5 shows the biomass production in media R1, R2 and the control medium BOLD.

There was growth of *Chlorella* sp. in both R1 and R2 media, reaching maximum concentrations of 0.53 ±0.046 and 0.83 ±0.021 g·dm^{-3}, respectively. In this work, it was found that the *Chlorella* sp. strain was able to reach biomass concentrations above the control in R2, which can be explained by the concentrations of nitrate, phosphate and organic compounds present in R2. Microalgae have been cultivated in an autotrophic way, using their photosynthetic capacity, and it has been found that some microalgae are able to grow in heterotrophic and/or mixotrophic conditions, and others may have a sequential or simultaneous mixed metabolism [Florez *et al.* 2017]. This phenomenon of mixed metabolism usually occurs in cultures in wastewater, photosynthesis process, which is influenced by light intensity, and respiration (assimilation of organic substrate), and they occur simultaneously [Ebrahimian *et al.* 2014]. Therefore, ATP formed from the photochemical reaction enhances organic

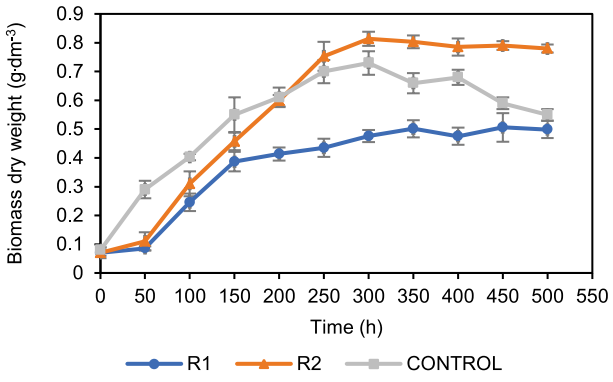

Fig. 5. Biomass production in different media: R1 = raw wastewater, R2 = treated wastewater, and control; source: own study

substrate anabolism and increases growth in wastewater [Kong 2011], as evidenced in this work.

Figure 6 shows *COD*, nitrate and phosphate removal efficiencies for each of R1, R2 and the control media in the activated sludge (A.S.) system. It was found that R2 and the control reached *COD* removal rates above 85%, which shows that both *Chlorella* sp. in R2 and microorganisms are present in the A.S. In R1, removal rates of 52% were achieved. Regarding the nitrates load, treatment where microalgae grew reached a removal rate of 98%, while in the activated sludge system, the concentration of nutrients increased considerably, which is explained by the fact that in an aerobic system, ammonia and ammonium salts are oxidised to nitrate. Finally, the phosphates removal by microalgae in both effluents was 81% for R2 and 78.5% for R1, both were above the control. It was found that *Chlorella* sp. has the ability to assimilate organic substances present in wastewater. Several authors have shown that microalgae of the Chlorophyta division have the ability to assimilate organic substrates and grow in mixotrophic or heterotrophic conditions [Leal Medina *et al.* 2017]. In a similar vein, cultivation conditions influence biomass production and accumulation of metabolites. The characteristics of an organic substrate growth allows for a lower energy expenditure and the growth does not depend on light compared to photoautotrophic processes, whereas cell density increases in the process of self-shading, which limits growth despite the existence of nutrients in the medium [Fernandez *et al.* 2013; Rosenberg *et al.* 2008]. In this work, this process was evidenced as shown in Figure 5. This enables the removal of organic substances, nitrogen, and phosphorus compounds.

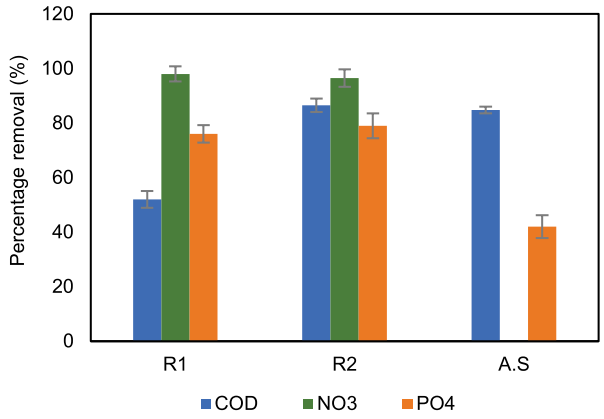

Fig. 6. Chemical oxygen demand (*COD*), nitrate and phosphate removal; R1 = raw wastewater, R2 = treated wastewater, A.S. = activated sludge; source: own study

CONCLUSIONS

This work shows a monitoring process used to determine the flow and physicochemical characteristics of domestic and wastewater effluents from a farm located in eastern Colombia. The effluents are used as a medium for microalgae cultivation. Results obtained after four months of study show that the average flow rate ranges between 0.18 and 0.30 $dm^3 \cdot s^{-1}$, and the treatment performed leads to a reduction of acidity, hardness, *TDS*, salinity, and sulphate content. Although the characteristics of the wastewater improved, its conductivity has been 231.60 $\mu S \cdot cm^{-1}$. Thus, it is recommended to examine water generated in the first hours of the day, between 8:00 and 9:30 a.m., as it has the highest concentration of pollutants in the form of fats and oils. Nitrate concentration increased (from 46.63 to 225.21 $mg \cdot dm^{-3}$) and phosphate concentration decreased slightly (from 9.65 to 6.21 $mg \cdot dm^{-3}$). Finally, the study has found that organic substances present in the wastewater do not affect the growth of microalgae. This demonstrates their potential not only as bioremediation agents for this type of water, but also their biotechnological potential for obtaining metabolites, such as lipids, carotenoids, and proteins.

ACKNOWLEDGMENTS

The authors thank Universidad de Cartagena and Universidad Francisco de Paula Santander (UFPS) for providing the equipment and software necessary to successfully complete this research.

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