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Biological and photocatalytic treatment at pilot plant scale of synthetic coloured wastewater produced in university teaching laboratories

Tratamiento biológico y fotocatalítico a escala de planta piloto para aguas residuales coloreadas sintéticas producidas en laboratorios de docencia universitaria

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Abstract

Dyes and chemical additives, present in wastewater produced in Microbiology teaching laboratories generate high contamination. They are difficult to degrade and according to the Colombian regulation should be treated before final disposal. In this work, a pilot plant was used to treat synthetic wastewater (SWW) containing a mix of dyes (10 mg/L Crystal Violet, 10 mg/L Congo Red, 10 mg/L Malachite Green and 10 mg/L Fuchsin Gram). To remove this type of contaminants initially, the capacity of a fungal/bacterial sludge (FBS) on a 1 L scale was determined. Subsequently, the operating conditions that favor tertiary treatment were selected by using TiO_2/UV photocatalysis at 0.25 L scale and finally the complete treatment plant was integrated and evaluated (Pre-treatment, secondary treatment and tertiary treatment). The SWW that entered the plant had the following characteristics: Chemical Oxygen Demand (COD): 4033 mg/L, Biochemical Oxygen Demand (BOD_5): 2750 mg/L, Color Units (CU): 2010, BOD_5/COD ratio: 0.68, 19±3 ºC and a pH of 7.0. After 37 hours of plant operation, overall removal was 98 ± 3%, 99 ± 6% and 99 ± 8%, for COD, BOD_5 and CU. Demonstrating that integration of unitary operations in a sequential and orderly manner, are a novel alternative for treatment of this type of wastewater.

Keywords: Synthetic wastewater, pilot plant, pre-treatment, secondary biological treatment, tertiary photocatalytic treatment.

Resumen

Los colorantes y aditivos químicos, presentes en el agua residual producida en los laboratorios de docencia en Microbiología, generan una contaminación elevada, son difíciles de degradar y según la normatividad colombiana deben ser tratados antes de realizar su vertimiento. En el presente trabajo se implementó una planta piloto para el tratamiento de un agua residual sintética (ARS) que contenía una mezcla de colorantes (Cristal violeta 10 mg/L, rojo congo 10 mg/L, verde de malaquita 10 mg/L y fucsina de Gram 10 mg/L). Inicialmente, se determinó la capacidad de un lodo fúngico/bacteriano (LFB) para remover este tipo de contaminantes a escala de 1 L. Posteriormente se seleccionaron las condiciones de operación que favorecen el tratamiento terciario empleando fotocatálisis con TiO_2/UV a escala de 0.25 L y finalmente se integró y evaluó la planta de tratamiento completa (Pre-tratamiento, tratamiento secundario y tratamiento terciario). El ARS que ingresó a planta tenía las siguientes características: Demanda química de oxígeno (DQO): 4033 mg/L, demanda bioquímica de oxígeno (DBO5): 2750 mg/L, unidades de color (UC): 2010, relación DBO_5/DQO: 0.68, 19±3 ºC y un pH de 7.0. A las 37 horas de operación de la planta las remociones globales fueron del 98±3%, 99±6% y 99±8%, para DQO, DBO_5 y UC. Demostrando que la integración de operaciones unitarias de forma secuencial y ordenada, son una alternativa novedosa para el tratamiento de este tipo de aguas residuales.

Palabras clave: Agua residual sintética, planta piloto, pre-tratamiento, tratamiento biológico secundario y tratamiento fotocatalítico terciario.

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1 Introduction

Water resources in Latin America are considered one of the most valuable riches on the continent. Thanks to this privilege and its distribution across the territory, it is possible to establish a range of specific productive processes, depending on the needs of each country, region and area (Hernández-Salazar *et al*., 2017). These productive systems can be classified as agricultural, livestock, manufacture, public service providers and industry sectors (Campos and Saconi, 2014; Ministerio de Ambiente y Desarrollo Sostenible, 2015). Each of these sectors use water resources in different proportions depending on their production needs, transforming it from drinking water to wastewater with different chemical, physical and biological characteristics (Weber and Campos, 2016; Crini and Lichtfouse, 2019a). Therefore, this wastewater needs to be treated before being disposed into sewage systems and then into water bodies (Gaviria *et al*., 2018; Pedroza *et al*., 2018).

Hospitals, universities and research centers are sectors providing services to the community. In universities with a School of Biological Sciences, research and teaching laboratories are essential for students' vocational education (Fernández *et al*., 2015). It is possible that these laboratories produce solid, liquid and gaseous waste, which change according to the academic period, as well as the type of courses taught in different semesters (Hernández *et al*., 2020). Of the three types of waste, liquid waste or wastewater produced from prepared biological stains to observe eukaryotic and prokaryotic cell morphology and structure, has a great environmental impact. These stains have high contaminated charges associated with chemical oxygen demand (COD), ranging between 2000 and 9920 mg/L, Biochemical Oxygen demand on the fifth day (BOD_5) with values between 730 - 3696 mg/L, total suspended solids (TSS) with values between 1.0 and 400 mg/L, Color units (CU) with values between 1006 - 2640 CU and Escherichia coli colony forming units (CFU/mL) with values between $1 \times 10^5 - 1 \times 10^8$ CFU/mL (Hernández *et al*., 2020). Due to their chemical complexity, they are considered as hazardous wastewater and are handled by a contractor for disposal. Although, this management is satisfactory, certain volumes could be discharged through the sewage system, causing adverse effects on the aquatic ecosystem. To prevent this, a simple treatment alternative to implement in enclosed spaces, such as university laboratories or investigation centers, should be considered (Hernández *et al*., 2018; Pedroza *et al*., 2018).

For treatment of this type of liquid waste, various physical and chemical technologies are available, which involve different processes, such as coagulation-floculation (Jafari *et al*., 2012), filtration, nano-filtration, micro-filtration (Jafari *et al*., 2012), advanced oxidation (Fernández *et al*., 2016; García *et al*., 2017) and chemical precipitation (Moreira *et al*., 2017; Crini and Lichtfouse, 2019b). All of these technologies are efficient in removing most contaminants. However, high organic charges and dyes can substantially decrease the removal percentage, where the effluent would not meet 100 % with the current environmental regulation (Ministerio de Ambiente y Desarrollo Sostenible, 2015). Resulting in suspension of the contractor's environmental licenses and permits (Ministerio de Ambiente y Desarrollo Sostenible, 2015), and affecting organisms in aquatic ecosystems (Moreira *et al*., 2017). Therefore, development and integration of technologies; alternating physical, chemical and biological processes are sought to improve efficiency removal, decrease operation times, diminish solid sub-products with low biodegradability and reuse the final effluent or recover bio-products with increased added value (Guo *et al*., 2014; Barrera *et al*., 2014; Pedroza *et al*., 2018; Diaz-Elsayed *et al*., 2019).

In conventional treatment plants, first physical or chemical process are implemented to remove solids from the wastewater, homogenise initial contaminant load, adjust pH and if necessary, reduce the temperature (Pedroza *et al*., 2018). Thus, the wastewater is conditioned to continue with biological or secondary treatment (Hernández-Salazar *et al*., 2017; Crini and Lichtfouse, 2019ab). To achieve a certain degree of effluent quality, the biotechnological potential of microorganisms of different trophic levels is used, reducing the initial contaminant load to a certain level in a secondary treatment (Pedroza *et al*., 2007; Ardila-Leal., *et al*., 2020; Machineni, 2019).

Currently, use of heterotrophic bacteria (Hernández *et al*., 2020), photoautotrophic (Machineni, 2019), filamentous fungi (Badia-Fabregat *et al*., 2017), yeast and microalgae (Ardila-Leal *et al*., 2020) are highlighted, which create consortia and highly specialized communities to potentiate biotransformation of dyes, emerging contaminants, organic matter, tense active substances, among others (Chen *et al*., 2017; Maddela *et al*., 2019). These communities remove contaminants by a range of

mechanism, such as adsorption to cell wall and production of exopolysaccharides that act as organic adsorbents (Anderson *et al*., 2011; Machineni, 2019). Additionally, they can biotransform certain compounds by reductive and oxidative enzymatic systems (Badia-Fabregat *et al*., 2017; Najera *et al*., 2018; Méndez-Loera, 2019).

To complement the mediated action by bacteria, it is possible to incorporate to these consortiums, white rot fungi, also called ligninolytic fungi (Rivera *et al*., 2013; Pedroza *et al*., 2018; Martínez-Sánchez *et al*., 2018). These fungi have the capacity to remove contaminants by adsorption mechanisms to fungal cell walls and a wide range of low specificity extracellular enzymes that biotransform contaminants through demethylation, hydroxylation, oxidation and aromatic ring breakdown (Rojas *et al*., 2016; Morales *et al*., 2016; Blanco *et al*., 2018). Among the most representative enzymes are Laccases (EC 1.10.3.2), Manganese peroxidases (EC 1.11.1.13) and Lignin peroxidases (EC 1.11.1.14) (Rivera *et al*., 2013; Rivera *et al*., 2018; Martínez-Sanchez *et al*., 2018; Méndez and Loera 2019; Ardila-Leal *et al*., 2019; Ardila-Leal *et al*., 2020). Once the secondary microorganism treatment is finished, the effluent can pass to a tertiary treatment to further improve the quality. Advanced oxidation processes (AOPs) are among the current successful technologies evaluated (Harrelkas *et al*., 2008; Zangeneh *et al*. 2015; Torres-Segundo *et al*., 2019; Suarez-Vázquez *et al*., 2019). In general, AOPs are defined as a set of physico-chemical processes specifically designed to generate reactive oxidative species, such as (OH^*) that concomitantly help with in situ oxidation processes. Hydroxyl radicals can be generated by different ways: i) Chemical oxidation methods using hydrogen peroxide/ozone or the Fenton process. ii) Gamma rays, electrons, UV radiation or ultrasound direct interaction methods iii) photocatalytic processes, in which the production of radicals from the catalyst (semiconductor oxide) occurs in the presence of UV/Vis (Campos and Saconi, 2014; Zangeneh *et al*., 2015; Weber and Campos, 2016; Márquez-Ramirez *et al*., 2019; Morones-Esquivel *et al*., 2020).

In particular, combination of biological and photocatalytic processes represents a novel alternative for liquid waste treatment with high loads of organic matter and color, as microorganisms decrease initial concentrations to continue with the photocatalytic process and complete the mineralization until CO_2, dissolved ions and water are produced (Harrelkas *et al*., 2008; Jafari *et al*., 2012; Natarajan *et al*., 2018). One restriction of this technology is that removal efficiency decreases, if wastewater has high concentrations of a determinate contaminate and high quantities of dyes. Essentially, because there is saturation of the active sites on the surface of the semiconductor and ultraviolet light does not reach the surface of the semiconductor to generate the photoexcitation process (Pedroza *et al*., 2007; Blanco *et al*., 2018; Waghmode *et al*., 2019; Ceretta *et al*., 2020).

Therefore, the objective of this study was to implement and evaluate a pilot plant for 37 h, which integrated a secondary process with fungal/bacterial sludge (FBS), and a tertiary process with photocatalysis (TiO_2/UV), to treat synthetic wastewater (SWW), similar to that produced in investigation and teaching laboratories.

2 Materials and methods

2.1 Microorganisms used and fungal/bacterial sludge production

Fungi *Pleurotus ostreatus* (CMPUJH124), *Trametes versicolor* (CMPUJH125), and Bacteria (*Pseudomonas fluorescens* CMPUJ 376, *Pseudomonas azotoformans*, *Enterobacter xianfangensis* and *Bacillus subtilis* CMPUJ 075) were obtained from the microbial collection at Pontificia Universidad Javeriana, Bogotá, Colombia. Microorganisms were reactivated and inoculums were produced according to the methodology reported by Pedroza *et al*., 2018; Hernández *et al*., 2020. To produce, fungal/bacterial sludge (FBS), a 1:1 microorganism consortia mixture was made from *P. ostreatus* and *T. versicolor* pelletized cultures. To this pelletized biomass 2 mL of bacterial consortia (1.0×10^6 CFU/mL) was added, which was homogenized using an electric blender for two minutes. Before initiating with the pilot plant experiments, the FBS was stored at 4 ºC in a 2.0 L of SWW solution, and feedings were performed every 4 days with fresh SWW to favour FBS adaptation and stabilization to the contaminant mixture. Additionally, 1 L scale experiments were performed containing only 1:1 *P. ostreatus* and *T. versicolor* pelletized biomass (fungal sludge, FS), which were stored under the same conditions as FBS.

2.2 *Synthetic wastewater preparation and characterization*

Synthetic wastewater (SWW) was made of Crystal Violet (10 mg/L), Congo Red (10 mg/L), Malachite Green, (10 mg/L) and Fuchsin Gram stain (10 mg/L). The dyes were mixed in 1:1:1:1 proportion. Glucose (0.5 mg/L), universal peptone (0.05 mg/L), Copper Sulfate (7.5 mM), Manganese Sulfate (1.0 mM) and phosphate di-acid of potassium (0.1 mg/L), were also added for the synthetic wastewater preparation (Hernández *et al*., 2020).

To determine the wavelength at which the greatest absorption occurred, a spectral curve UV/Vis (200-800 nm) using a Genesis 20® spectrophotometer was made. The selected wavelength was used to quantify color units (CU) after the sample was centrifuged at 9,000 x g and 4 ºC for 10 minutes, employing a refrigerated Sorvall® centrifuge. Color units (CU) were determined using Equation 1 (Livernoche *et al*., 1983).

$$CU = \frac{A_1 \times 500}{0.132} \quad (1)$$

where: A_1 wavelength of SWW at a maximum absorbance and 0.132 represents 500 CU of platinum-cobalt standard solution measured at $ABS_{546\,nm}$ (Livernoche *et al*., 1983).

Because the dyes have different chemical composition they absorb light at different wavelengths. For example, Crystal Violet absorbs at 589 nm, Malachite Green at 619 nm, Fuchsin Gram stain at 546 nm and Congo Red at 486 nm. The 1:1:1:1 stain mix generated a signal at 546 nm. This wavelength was used to calculate Color Units (CU). Additionally, Chemical Oxygen Demand (COD) (mg/L) was determined employing the closed reflux method using the HACH® commercial kit (method 8000), (Jirka and Carter, 1975). For the Biological Oxygen Demand at day five (BOD_5), (mg/L) Velp Scientifica® respirometric bottles were used (Pedroza *et al*., 2018). Total suspended solids (TSS) were determined by using the 2540 D method of Standard Methods for the Examination of Water and Wastewater (Rice, 2017). pH was determined using an Ohaus® pH meter. COD, BOD_5 and CU were converted into removal percentage by using equation Eq. 2.

$$Removal\,(\%) = \frac{Initial\ value - Final\ value}{Initial\ value} \times 100 \quad (2)$$

where: Removal of COD. BOD_5 or CU (%) is the removal percentage of the analyzed variable (Leal-Ardila *et al*., 2020).

2.3 *Removal experiments by employing fungal bacterial sludge at 1 L scale*

The COD and CU removal by the fungal bacterial sludge (FBS) employing SWW was performed at 1 L scale with 80% effective work volume (v/v). To this end, 720 mL of SWW at pH of 7.0 and 80 mL of FBS were added. Each reactor had a port for sampling, another for air inlet and another for air outlet. A 5 W air pump provided diffuse aeration (0.5 L/min). Samples were collected at the beginning and every 24 hours until 72 hours were completed to determine COD and CU removal as a function of time. Additionally, removal capacity of fungal sludge (FS) without bacteria was evaluated using a series of three 1 L reactors that only contained *P. ostreatus* and *T. versicolor*. The operational conditions were the same than for FBS. Results obtained after 72 h were used to establish significant differences between FBS and FS by an analysis of variance (ANOVA) with a 95% confidence interval. SAS® software was employed for analyses.

2.4 *Condition selection favoring TiO_2/UV photocatalytic treatment*

Previous to the plant pilot assembly (primary, secondary and tertiary treatment) two independent experiments were carried out to select which conditions favored TiO_2/UV photocatalytic treatment.

2.4.1 *TiO_2 selection*

Four types of commercial TiO_2 were evaluated: Sigma-Aldrich™ Titanium Dioxide: Anatase/Rutile (https://www.sigmaaldrich.com, 2020). Titanium Dioxide Degussa P-25: Anatase/Rutile (https://www.nanoshel.com/product/degussa-p25-titanium-dioxide, 2020), Titanium Dioxide USP Anatase (https://www.paginasamarillas.com.co/empresas/quimicos-de-la-13-con-13, 2020) and Titanium Dioxide USP Anatase (www.quimicoscampota.com, 2020). Sigma-Aldrich and Degussa P25 TiO_2 are semiconductor oxides with high percent purity (99.5% Sigma-Aldrich and 94% Degussa P25). Both have been used in various photocatalytic studies (Dnyaneshwar *et al*., 2017; Muniandy *et al*., 2016). In relation to TiO_2 USP

from "Quimicos de la 13" and "Quimicos Campota Ltda", its purity was 90%. It is used in Colombian pharmaceutical and petrochemical industry as the pro-oxidant additive for Oxo degradable plastic (Blanco *et al*., 2018; Rivera *et al*., 2018; Gómez, 2018).

For these assays, 0.25 L quartz reactors were fabricated using 15 cm long x 20 cm height x 4 cm (thick) plates. Each reactor had an air inlet, where a 5.0 W air pump (0.1 L/min) provided diffuse aeration. Moreover, each reactor contained 200 mL of untreated SWW and 2% TiO_2 (m/v) of each type. Initial concentrations were 3050 ± 98 mg/L COD, 2145±121 CU and a pH of 5.4, assays were performed in triplicate. Quartz reactors were placed inside a 60 x 50 x 30 cm galvanized box, which contained 2 UV lamps: 15 W (254 nm) UVA-340 lamp. Each lamp was placed on each side of the reactor at a distance of 10 cm from the base of the galvanized box and 5 cm from the reactor. After 6 hours of treatment analyzed response variables were pH, CU removal and COD (Blanco *et al*., 2018). To determine significant differences among the types of TiO_2 an ANOVA with a 95% confidence interval was performed using the SAS® software.

Furthermore, two additional analyses were performed to the selected TiO_2: TiO_2 crystalline phase analysis by X-ray diffraction (XRD) using PANalytical X'Pert PRO MOD diffractometer, operated with Cu Kα radiation (wavelength = 1.54050 Å) at 45 kV and 40 mA. XRD patterns were collected in 2θ range from 10 to 90º with a scan rate of 0.026º per 40 s and a step size of 0.026º. In addition, scanning electron microscopy (SEM) was performed employing a microscopic Jeol^{TM} JSM 6490LV with a 10 kV to 20 kV potency SEI signal and 10000 X. Samples were coated with gold in Denton Vacuum Desk IV preparation system.

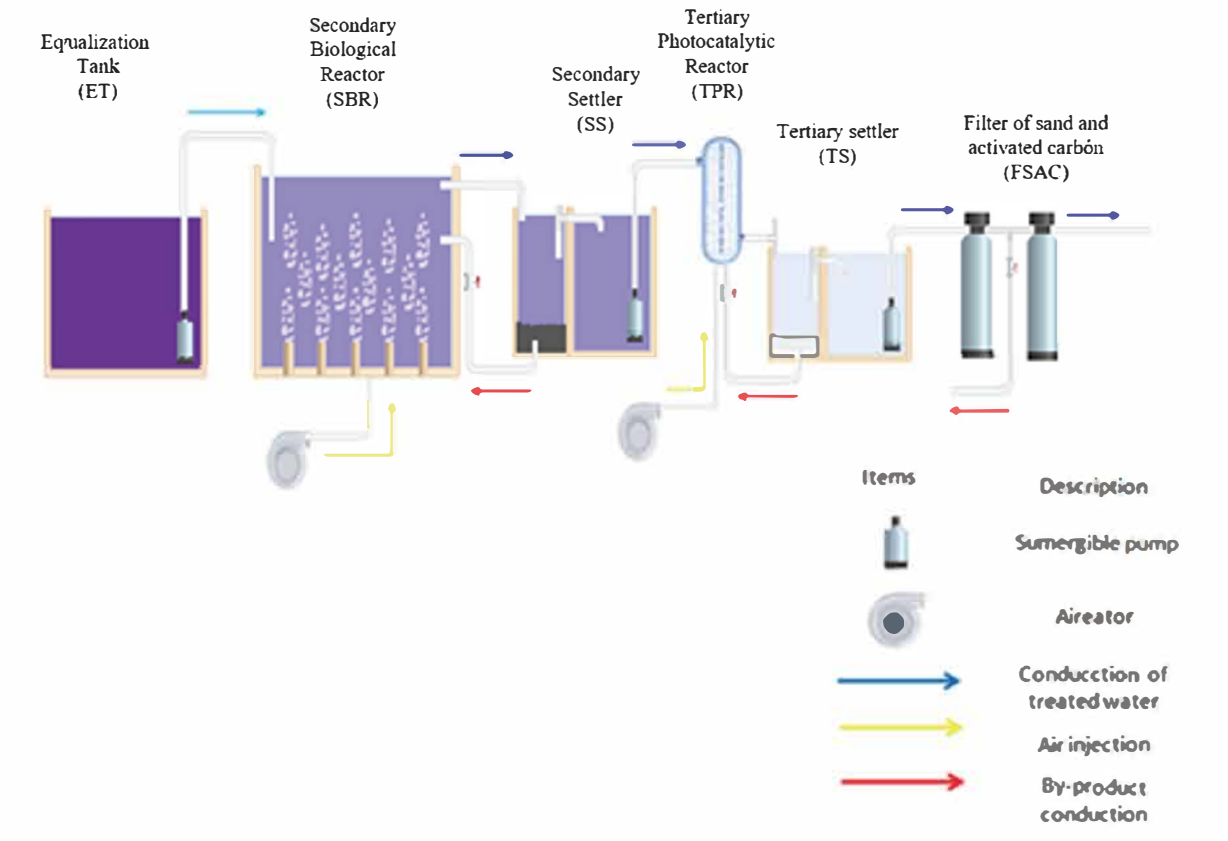

Fig. 1. Schematics of Pilot Plant. The Pilot Plant is composed of an Equalization Tank (ET), Secondary Biological Reactor (SBR), Secondary Settler (SS), Tertiary Photocatalytic Reactor (TPR), Tertiary Settler (TS) and Filters of sand and activated Carbon (FSAC).

2.4.2 2^2 *factorial design with central points*

A 22 factorial design with three central points was performed to evaluate the effect of COD concentration. Factor A: COD concentration with a (-1) level at 1170 mg/L and a (+1) level at 4500 mg/L. Factor B was TiO_2 in g/L, with a (-1) level of 0.5% (m/v) and a (+1) level of 1.0% (m/v). The design generated a total of four treatments (-1-1, +1-1, -1+1 and +1+1) and three central points (0,0). The experiments were performed in the same reactors evaluated in the previous section. Initial and final samplings (6 hours) were carried out to determine the pH and CU removal. The selection of the significant treatment was determined by an analysis of variance and a comparison of means using Design-Expert® and SAS® software.

2.5 *Synthetic wastewater test at pilot-level scale*

The pilot treatment plant consisted of a 16 L equalization tank (ET), a secondary biological reactor (SBR) of active sludge modified with an 11 L extent aeration, a 30 L secondary settler (SS), a 2.6 L tertiary photocatalytic reactor (TPR), 40 L tertiary settler (TS) and two 2.6 L filters (sand and active carbon, SACF) (Fig. 1). Gravity was the main force driving SWW through the plant, although a 28 W 10 - 120 V/60 Hz voltage submergible pump was used. Synthetic wastewater flow from the equalization tank to the biological reactor was feasible by one of the submergible pumps. From the SBR to the SS gravity was used. From the settler to the photocatalytic reactor, again a submergible pump was used, and last flow to the tertiary settler and filters was by gravity. The retention time in each unit was initially 30 minutes in the equalization tank, 24 hours in the biological reactor, 3.5 h in the secondary settler, 6 h in the photocatalytic reactor, 2 h in the tertiary settler and 1 h in the filters which ended the system, for a total of 37 h. Samples were collected at the beginning, at the end of the secondary settler, at the end of the tertiary settler and at the end of the filters, in order to determinate pH, CU removal, COD and BOD_5.

Three types of analyses were carried out with the experimental data. Removal percentage was calculated taking into consideration the concentrations that came out from each unit, to estimate the partial efficiency. The second analysis calculated was total plant removal for each parameter, taking in consideration the initial values (equalization tank) and the values obtained at 37 hours of treatment. Finally, the results were analyzed as a function of the concentrations in mg/L to observe their diminishment between units (Pedroza *et al*., 2018; Blanco *et al*., 2018).

3 Results and discussion

3.1 *Synthetic wastewater characterization*

The dyes used to prepare biological stains were from chemical origin and had aromatic structures with different chemical substitutions to favor their absorption by cellular structures. The color in Crystal Violet, Fuchsine Gram and Malachite Green was determined by the chromophore group to which Triphenylmethane was associated with the aryl group. In contrast, in Azo-dyes (Congo Red) the chromosphere (azo group) is made of amino ($-NH_3$), carboxyl ($-COOH$), sulfonate ($-SO_3$) and hydroxyl groups ($-OH$) (Morales *et al*., 2016b; Marquez-Ramirez *et al*., 2019; Suarez-Vázquez *et al*., 2019).

The presence of these compounds increased initial COD concentration with values of 2633 ± 200 mg/L. Conversely, BOD_5 concentration was low 345 ± 17 mg/L. The treatability ratio BOD_5/COD was 0.130 ± 0.014, revealing that dyes and their low biodegradability greatly contributed to the contaminating loads. Therefore, a fungal and bacterial consortium was developed and evaluated to improve the biological treatment. The aim was to further decrease removal percentage and even remove toxic intermediaries of the initial colorants that may remain if only bacteria are employed (Deveci *et al*., 2016). CU was 2133 ± 165, SST 1044 ± 101 mg/L and pH 6.0 ± 1. The results herein obtained were similar to those reported by others authors. In their studies it was observed that a mix of dyes increased COD concentration and treatment in a short period of time was difficult (Fernández *et al*., 2015; García *et al*., 2017; Suarez- Vázquez *et al*., 2019; Hernández *et al*., 2020).

3.2 *Removal experiments employing Fungal Bacterial Sludge at 1 L scale*

For these experiments at 1.0 L scale, the initial concentrations were 2,633 ± 200 mg/L, 345 ± 17 mg/L, 1044 ± 101 mg/L, 2133 ± 165, for COD, BOD_5, SST, CU and pH 7.0 ± 1, respectively. Fungal bacterial sludge (FBS) and fungal sludge

(FS) results at 1 L reactor during a 72 h process are presented in Fig. 2. A slight decrease in pH during the first 48 h (5.8 FBS and 6.7 FS) for both experiments was observed, which could be related to glucose consumption that was added to SWW as the carbon source. Microorganisms assimilating this carbon source through heterotrophic metabolism produced organic acids that decreased SWW's pH. In contrast, from 48 to 72 hours an increase in pH was observed, which ended at 7.1 and 6.85 for FBS and FS, respectively (Fig. 2A). This slight increase could be attributed to the production of intermediaries, such as ammonium, which is produced from organic nitrogen mineralization (universal peptone) (Morales *et al*., 2016a; Suarez-Garcia *et al*., 2019; Hernández *et al*., 2020). Some authors report that addition of a carbon source and simple nitrogen, favors dye and aromatic compound biotransformation. The presence of a co-substrate in wastewater allows for the microorganism to develop and maintain the primary metabolism, while the aromatic structures are modified. At the same time, they can obtain carbon and energy from them (Morales *et al*., 2016b; Rivera *et al*., 2018; Méndez-Loera, 2019).

According to the physical parameters, it was observed that FBS was significantly better in comparison with FS alone in TSS removal (initial value: 1,044 ± 101 mg/L) and CU (initial value: 2133±165). At 72 h obtained removal values of 64 ± 2% and 98 ± 5% for TSS and CU were determined ($p = 0.0078$ and $p = 0.002$) (Fig. 2B). In contrast, removal with FS was 50 ± 3% and 59 ± 5% for TSS and CU, respectively. Although, SST removal with FBS was satisfactory, the final concentration was still high (378 mg/L), suggesting the microorganisms, which formed the secondary sludge (fungi and bacteria) grew at the expense of organic matter contained in the SWW (initial values: 2633 ± 200 mg/L and 345 ± 17 mg/L for COD and BOD_5; final values: 790 mg/L and 120 mg/L for COD and BOD_5, respectively). Microorganism proliferation in the biological reactor is desirable and related with their capacity to biotransform pollutants present in the wastewater and subsequent treatments. However, in terms of treatment plant design and performance, part of those TSS and SS, should be eliminated to prevent an increase in COD and BOD_5 concentrations in the effluent, coming from the secondary treatment. This is achieved by incorporating another unit, such as a secondary sedimentation or a clari-floculator, allowing for the satisfactory elimination of sludge and part of the TSS (Suresh *et al*., 2018). In relation to color unit removal, adsorption mechanisms allow the dye to adhere to the fungal/bacterial wall (FBS) or only fungal cell wall (FS), which were possible in both experiments. This is a physical/chemical process, independent of the microorganism's metabolism. It occurs during the first hours of contact and it is strongly influenced by pH, which initiated with a value of 7.0 and started to decrease from hour 10 (Fig. 2). Under these acidic conditions, the cellular wall is negatively charged and favored cationic dye adsorption (Morales *et al*., 2016a; Morales *et al*., 2016b). Once the adsorption and desorption balance are obtained, the dyes are bio-transformed by enzymatic mechanisms that include enzymes such as polyphenol oxidases, peroxidases and peroxide generating enzymes, such as aryl alcohol dehydrogenases, glyoxal oxidases, among others (Rivera *et al*., 2013; Morales *et al*., 2017; Mendez and Loera, 2019).

The COD and BOD_5 removal was higher when FBS was employed (70±2% and 65±5%), in comparison with FS (55 ± 2% and 36 ± 3% for COD and BOD_5, respectively) (Fig. 2C). These results indicated that fungal and bacterial consortia exercised a positive interaction that increased removal percentage, if compared with two white rot fungi in the absence of bacteria. In the FBS experiments, fungi were possibly responsible to initiate the biotransformation of the most difficult degradation compounds, such as dyes. Subsequently, bacteria could use part of the simplest intermediaries to complement COD and BOD_5 removal (Badia-Fabregat *et al*., 2016; Badia-Fabregat *et al*., 2017). In the beginning of the process, in addition to the two microorganisms used, co-substrates were added to the SWW (glucose and universal peptone) to provide carbon and energy. Apart from that, another condition that favoured the contaminant's removal was that the FBS had already adapted to the dye mix, because the FBS was stored in a dissolved solution of SWW before the initiation of removal experiments at 1.0 L scale in the pilot plant.

The positive response to this adaptation was notorious when our results are compared with those previously published by Hernández et al, in 2020. In the Hernández' studies a similar SWW was used with the same microorganisms, yet they lacked a previous adaptation process. Only cultures were produced, the biomass was recovered and one-liter reactors, which contained SWW were inoculated, determining that the highest removals were obtained at 96 hours of treatment; 24 hours in addition to those obtained in this study (72 hours) (Hernández *et al*., 2020).

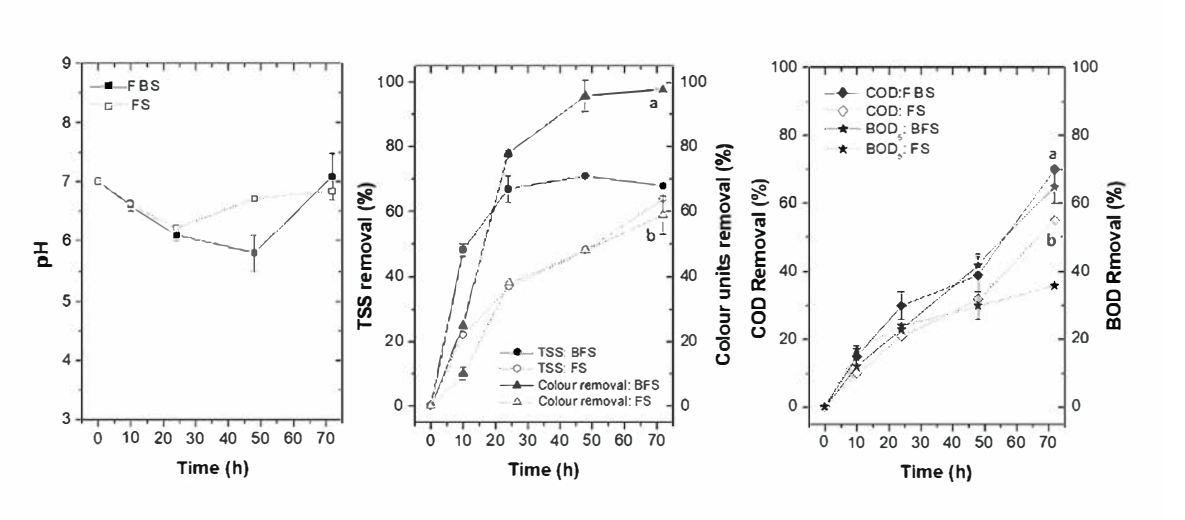

Fig. 2. (A) pH. (B) TSS and Color units removal (%). (C) COD and BOD_5 removal (%) for FBS and FS. The letter a, correspond to the best treatment obtained follow by the b and c letters (test of Tukey). The bars represent the standard divert and the average of three replicas.

3.3 Condition selection favoring TiO_2/UV photocatalytic treatment

3.3.1 TiO_2 selection

Experimental results to select TiO_2 to be used in the photocatalysis tertiary reactor are presented in Fig. 3A. For this experiment, COD's initial concentration was 3050 ± 98 mg/L, CU: 2145 ± 121 and pH of 5.4. Significant differences were observed among different types of TiO_2, where Sigma-Aldrich resulted in the highest removal percentage at 6 hours of process, with values of 58 ± 3% and 89 ± 5%, for COD and CU ($p = 0.004$ and $p < 0.0001$) (Fig. 3A). This type of TiO_2 has greater purity (99.5%) and the anatase phase proportion is higher than the rutile phase proportion (90:10). Hence, these two factors favoured SWW photocatalytic process with high COD and CU charges. The highest proportion of the anatase phase favoured CU and COD removal, because it is an indirect energy gap semiconductor with an of $Eg = 3.2$ eV value, suggesting the electron-hole pair was more stable than in the rutile phase and the recombination process was minor (Márquez-Ramírez *et al*., 2019; Morones-Esquivel *et al*., 2020).

On the other hand, with fewer impurities, the oxide semiconductor interacts with the dyes, resulting in more reactive oxygen species production. These, in term cleave the dye's chromosphere link groups and form aromatic intermediaries, such as benzene or naphthalene that require more treatment time to become aliphatic intermediaries or carry out complete mineralization to CO_2 (Márquez-Ramírez *et al*., 2019). Henceforth, COD removal was less in comparison with CU removal, as has been reported by other authors, such as Harrelkas *et al*., 2008 and Jafari *et al*., 2012. In their studies, they evaluated an isolated photocatalytic process in combination with a biological process, observing that by employing dyes of different chemical families, the first step carried out was chromosphere group oxidation, which absorbs light in the visible spectrum, decreasing the color level in the first minute of treatment. Therefore, COD concentration decrease is not directly proportional to CU removal (Harrelkas *et al*., 2008; Jafari *et al*., 2012).

The physical characteristics of Sigma's® oxide semiconductor is a white powder, with less than 5 μm in size that is easily dispersed in an aqueous solution. In acid and alkaline pH it tend to form aggregates with different forms (polymorphic) and sizes (polydisperse). Formation of these aggregates was observed at pH of 5.4 and was similar to the results obtained by Fernández *et al*., in 2015 and Gómez, 2018. These aggregates with different forms and sizes also could favour the photocatalytic process because they provide a larger surface area, with greater quantity of defects, superior capacity of initial contaminant absorption to the surface of the semiconductor, allowing elevated photocatalytic degradation efficiencies (Natarajan *et al*., 2018; Morones-Esquivel *et al*., 2020).

On the other hand, X-ray diffraction analysis determined the material's crystallinity. The most intense anatase phase orientation was 101 (Fig. 3C). Additionally, orientations 004, 200 and 211 from the same phase were observed. Rutile phase signals presented less intensity and 110 and 211 planes were observed. Results are in line with those reported by Sigma-Aldrich (Muniandy *et al*., 2016). The presence of those two phases allows for a more efficient dye oxidation, because the presence of both represents a higher redox potential, which is achieved by the transfer of electrons from the conduction band of the anatase phase to the conduction band of the rutile phase (Gómez, 2018).

3.3.2 2^2 Factorial design with central points

TiO_2 Sigma® 22 factorial design with three central point results are illustrated in Fig. 3D and Table 1. According to analysis of variance results it was observed that for CU removal the two factors, 48% COD initial concentration and 44% TiO_2, and their interaction had a significant effect on this dye removal ($p < 0.0001$ for the three). Associated with the contribution percentages of the evaluated factors, those with the highest hierarchy were COD initial concentration with 48% and the concentration of TiO_2 with 44%.

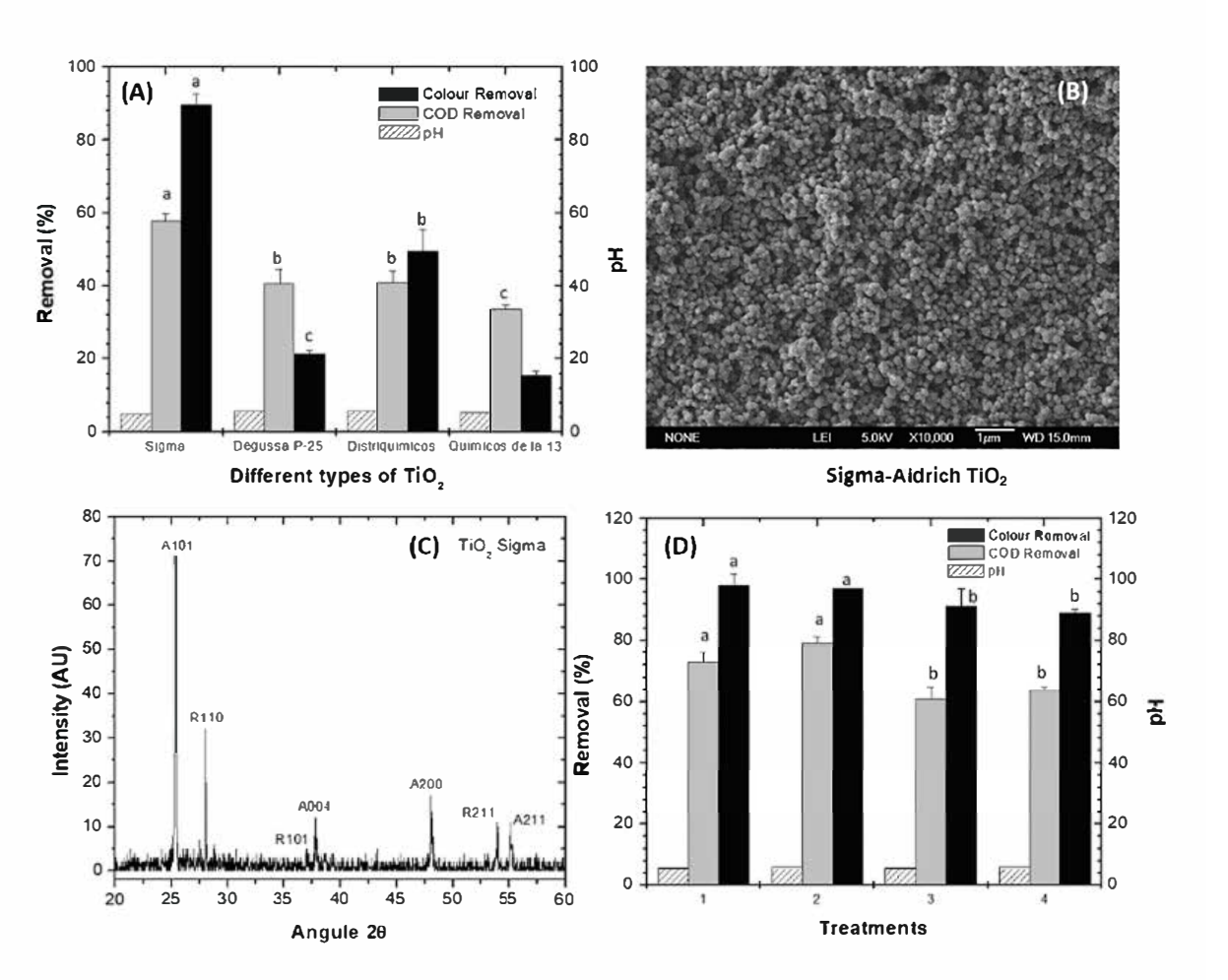

Fig. 3. Photocatalytic experiments (A). Selection of TiO_2. (B) Scanning electron microscopy of TiO_2 Sigma. (C) X-ray diffraction. (D). Factorial design 2^2 with three central points. The letters a, correspond to the best treatments to each type of experiment (Tukey test). The bars represent the standard deviation and the average of three replicas.

Subsequently, significant differences were observed when mean of treatments were compared (Fig. 3D). The greatest dye removal level was obtained for treatments 1 and 2, with percentages of 98 ± 2% and 97 ± 6% ($p < 0.0001$) at 6 hours of treatment. Likewise, no significant difference ($p > 0.0001$) between them was observed. Additionally, it was determined COD removal for those treatments were 73 ± 5% and 79 ± 2%, significantly higher values than those obtained in T3 (60 ± 3%) and T4 (64 ± 5%) ($p < 0.0001$). Based on these results, it was determined that the photocatalytic process was favoured under two conditions: The first one, by employing low COD concentrations and color units (initial values of COD 1,170 mg/L and 215 for CU), and the second one by employing high COD concentrations and low color unit concentrations (initial values of COD 4500 mg/L and 1143 CU). Because no significant differences between both treatments was observed, it was decided to continue with T2, which meant higher COD and CU concentrations, in an event that the biological reactor attached to the secondary sedimentation did not achieve high removal, then a tertiary treatment would be required to complete the treatment.

The grounds why COD and CU removal was favored could be established with the adsorption of some dyes to the semiconductor, which was influenced by SSW pH, favoring acidic pH (5.8). This pH value was below TiO_2's isoelectric point (pH of 6.5 ± 0.2), determining the semiconductor surface acquired a positive charge as a consequence of proton adsorption (Fernández *et al*., 2015). Under this condition, negative dyes are absorbed faster than positive charged ones. On the other hand, at this pH possibly the photogenerated h^+ could be very abundant, and by having higher oxidative power, they would be in part responsible for the dye induced photo oxidation (Fernández *et al*., 2015). Dyes with positive charge don't easily adsorb to the TiO_2 surface. Therefore, their oxidation could be carried out in part by hydroxyl radicals produced by water photolysis in the presence of ultraviolet light (Natarajan *et al*., 2018; Rivera *et al*., 2018; Marquez-Ramirez *et al*., 2019).

Contrarily, in this study initial COD concentrations didn't affect the photocatalytic process, and it was considered a favorable variable to the end result of the process. Various authors report that at high COD concentrations, it is possible that some organic matter present in the residual water can be adsorbed by the TiO_2 photocatalyzer. This in turn generates competence between ions and blocks TiO_2's active site, which affect the oxide-reduction process. Additionally, a screen process could be generated, and ultraviolet light would not be enough to influence the oxide component of the semiconductor, resulting in a reduced photo-excitation, where the same number of electron pairs would not be produced (Pedroza *et al*., 2007; Fernández *et al*., 2015; Blanco *et al*., 2018; Morones-Esquivel *et al*., 2020).

3.4 Synthetic wastewater test at pilot-level scale

Once FBS removal capacity in a 1 L reactor was evaluated, the type of commercial TiO_2 was selected, and conditions that favoured the photocatalytic process with light UV were determined.

Table 1. ANOVA results to 2^2 factorial design with three central points.

Colour Removal (%)			
Factor	**p value**	**Contribution (%)**	**Stand. Effect**
Model	< 0.0001		36.93
Factor A COD	< 0.0001	48	36.92
Factor B TiO_2 concentration	< 0.0001	44	21.77
Interaction AB	< 0.0001	8	21.78
R^2	0.996		
CV	0.189		
Adeq Precision	34		

The complete plant assembly was carried out, and its performance as a function of time was evaluated. In the beginning of the process, SWW characteristics when leaving the equalization tank (ET) were: pH: 6.0 ± 1, COD: 4033 ± 104 mg/L, BOD_5: 2750 ± 98 mg/L and CU: 2010±14.

In the ET, high COD, BOD_5 and CU removal were not observed. A pre-treatment was carried out, to adjust the pH so it would be within the range tolerated by fungi and bacteria. In this manner when the SWW entered the biological reactor, its growth and enzymatic activities would not be affected, generating a prolonged adaptation phase for FBS (Deveci *et al*., 2016). Authors such as Méndez and Loera reported in 2019 that among the great advantages of ligninolytic enzymes for pollutant biodegradation in wastewater were their low substrate specificity and wide pH range tolerance. Converting them in an attractive alternative for oxidation of contaminants that are difficult to degrade by conventional biological technologies in which only aerobic or anaerobic bacteria are used (Méndez and Loera, 2019), (Fig. 4A).

After 24 hours in SBR, removal percentage for COD (initial concentration 4033 mg/L) was 75%, BOD_5 (initial concentration 2750 mg/L) 75%, and CU (initial CU 2010) 45%, (Fig. 4A). FBS contact with SWW during the first hours, and thanks to the extended aeration that kept FBS in suspension, dyes could be absorbed to cellular walls of bacteria and fungi (Blanco *et al*., 2018; Hernández *et al*., 2020). This is the first mechanism concerned with SBR dye removal, which is independent of the microorganism's metabolism and is strongly influenced by SSW's pH (7.0 ± 3) (Martínez-Sánchez *et al*., 2018). Subsequently, it was possible to initiate stain biotransformation by chromophore group modification. To which, fungal extracellular ligninolytic enzymes (Laccase, MnP and LiP) and bacterial enzymes, such as peroxidases and phenol oxidases, among others participate (Pedroza *et al*., 2018; Martínez-Sánchez *et al*., 2018). Dye biotransformation allows for intermediary formation that can continue with the process of partial or complete mineralization to CO_2, fungal proliferation and bacterial biomass production.

However, stains use a single carbon source that may involve extended periods of adaptation, which can be reflected in SBR treatment time. To avoid this, simple co-substrates are used to favor fungi and bacteria primary metabolism, which are supplemented at low concentrations to avoid increasing organic matter content, expressed as COD and BOD_5. Thanks to this, in this work initial of COD, BOD_5 and CU concentrations were reduced. The use of co-substrates to favour pollutant degradation with complex chemical structures has been reported in both aerobic and anaerobic processes. Martínez-Sánchez *et al*. (2018) used Trametes versicolor immobilized in polyurethane foam for reactive black 5 dye discoloration and used Rhada mineral medium, demonstrating a discoloration greater than 84%. As a product of mineralization CO_2 was produced. Moreover, laccase activity increased in the presence of reactive black 5 dye.

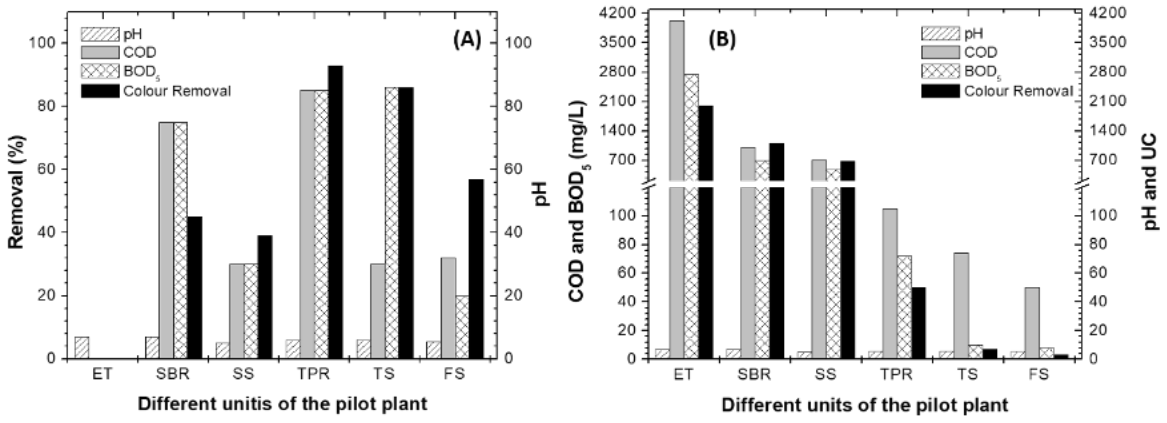

Fig. 4. Treatment plant performance for 37 hours. (A) Removal of parameters in percentages depending on the treatment units. (B) Decreasing of response variables.

In regard to anaerobic processes in 2010 Duran-Hinojosa *et al*., evaluated oxygen and glucose effect on the methanogenesis and mineralization of polymeric resins. The authors observed that glucose presence did not improve polymeric resin elimination. However, it did favour methanogenic activity, suggesting that glucose's presence may improve methanogenic sludge tolerance to dissolved oxygen and increase methane production, when compared to experiments where polymeric resins were used as the sole carbon source (Duran-Hinojosa *et al*., 2010).

Growth of fungal and bacterial biomass (FBS) inside the reactor increases as a function of treatment time. Hence, to prevent COD and BOD_5 concentrations from rising again they must be eliminated. By transferring FBS to the sedimentation tank and halting the extended aeration, biomass' dense flocs were formed that settled and moved onward to the interior of the sedimentation tank (SS). Additionally, part of the suspended solids and dissolved solids adhered to the flocs, determining that in this unit 30% of COD, BOD_5 and 38% of CU were additionally removed within two hours of sedimentation (Fig. 4A). Mareai *et al*. (2020), evaluated phenol, ammonium and COD removal present in pharmaceutical wastewater, employing an active sludge reactor and an extended aeration reactor. The authors observed that by adding small doses of activated carbon, secondary sedimentation and contaminant removal were improved. Demonstrating the importance of the sedimentation unit for the recovery of sludge to obtain a clarified effluent.

In the present investigation, despite obtaining a significant COD, BOD_5 and CU decrease within the biological reactor attached to the secondary settler, final values were still not within the general requirements for disposal of treated wastewater in Colombia (Ministerio de Ambiente y Desarrollo Sostenible, 2015). Therefore, the tertiary photocatalytic reactor (TPR) was incorporated into the treatment plant to aid with the removal of recalcitrant organic matter, which was not removed in the biological reactor or the secondary settler (705 mg/L and 481 mg/L for COD, BOD_5, and 678 CU, respectively).

This organic matter is made up of aromatic or aliphatic intermediates that are not susceptible to oxidation by biological processes, due to their redox potential. Thus, they require TiO_2/UV photocatalytic processes, where reactive oxygen species with high oxidizing power and low selectivity for contaminants or recalcitrant organic matter are produced. Therefore, COD and BOD_5 removal was favoured, with final values of 105 mg/L and 72 mg/L, respectively. Likewise, CU decreased, obtaining 50 CU and a final pH of 6.0 at 6 hours of treatment was attained (Fig. 4A).

Taking into account the values entering the TPR were COD 105 mg/L, BOD_5 72 mg/L and 50 CU, high removal percentages were observed for COD, BOD_5 and CU (85%, 85% and 93%, respectively). Deveci *et al*., in 2016 also observed the photocatalytic membrane reactor (PMR) efficiently removed low COD concentrations (1125 ± 70 mg/L). Furthermore, by integrating them with a fungi membrane reactor (FMBR), removal of color and COD were greater than 90% with a processing time of 27 hours (24 hours for biological treatment and 2 hours for photocatalytic treatment), (Deveci *et al*., 2016).

A homogenous photocatalytic process requires the presence of the semiconductor TiO_2 in suspension, which must be eliminated from the tertiary effluent because it increases suspended solids and gives the effluent a turbid appearance and a whitish color. To eliminate TiO_2, COD, BOD_5 and CU two additional units were required: the tertiary settler (TS) and sand and active carbon filters (SACF). The two units eliminated TiO_2 and part of COD, BOD_5 and CU, which left the tertiary reactor (TPR) through different mechanisms. In the tertiary settler (TS) the effluent's pH was 6.0, value in an instability range, in the vicinity of TiO_2's isoelectric point (5.0 and 7.0), (Fernández *et al*., 2015). At pH 6.0, formation of TiO_2 aggregates was favoured, increasing the hydrodynamic size of the particles, allowing for faster settling in the TS. Together with the TiO_2 aggregates, part of the COD, BOD_5 and dye probably also adhered, accomplishing removal percentages of 29.5 for COD (74 mg/L) and 86% for BOD_5 (10 mg/L) and CU (7.0), (Fig. 4A).

Last, in the sand and activated carbon filtration units (SACF), part of the non-sedimented TiO_2 at pH 6.0 was removed with a small fraction of COD and BOD_5. The main mechanisms involved were adsorption to the filter constituents and retention by particle size. As the effluent flowed through the filters end values of 50 mg/L (32%), 8 mg/L (20%) and 3.0 (57%), for COD, BOD_5 and CU, with pH of 6.0 were observed (Fig. 4A).

As shown in Fig. 4A, removal percentages did not have an increasing trend as a function of passing through each unit, this is because the outgoing values were used, making the percentages between units to fluctuate. In Fig. 4B it is depicted how the plant decreased contaminant concentration. Furthermore, it

can be appreciated as that the SWW flowed through different treatment units, the concentration decreased, such as at the end of the process values were as follows: pH 6.0, COD 50 mg/L, BOD_5 8.0 mg/L and CU 3.0 (Table 2). Global removal results were: 98%, 99% and 99%, for COD, BOD_5 and CU, with a final pH of 6.0. Finally, a second operation cycle was performed under same conditions, and removal levels between units did not fluctuate more than 10% (Demonstrating that the plant obtained similar results during the first operation cycle). On the other hand, on the second assembly 90% of FBS was re-circulated through the biological reactor to start a new operation cycle (not shown data). Re-circulation of FBS, which was previously adapted in SWW during the production and storage phase (Materials and Methods), guaranteed a high removal after 37 hours of treatment in the new cycle at the operation plant.

The beneficial and combined effect of physical, chemical and biological processes to improve treatment of non-domestic wastewater produced by different industrial sectors has been successfully reported by several authors. Lotito *et al*., in 2012 demonstrated for textile wastewater treatment, removal efficiency increased when a sequencing batch biofilter granular reactor (SBBGR) and an oxidation with ozone system was implemented. In the ozone reactor, recalcitrant organic matter from the biological reactor was oxidized and the final effluent complied with Italian regulations (Lotito *et al*., 2012).

Ferreira-Rólon *et al*. (2014), proposed a novel pilot treatment plant that incorporated physical, chemical and anaerobic biological processes for the treatment of nejayote or wastewater produced during the nixtamalization process of corn flour in Mexico. The authors showed that by using a calcium sedimentation-precipitation sequence, anaerobic treatment, separation and use of biogas, the final effluent contained a COD concentration below 200 mg/L. The effluent could be discharged through a draining system or flow through an aerobic polishing process that can be used in irrigation of fields.

Deveci *et al*. (2016) coupled two types of membrane reactors for textile wastewater treatment. The first one was a fungal membrane bioreactor (FMBR) and the second one a photocatalytic membrane reactor (PMR). In Deveci's work, initial COD concentration was 1125 mg/L; textile wastewater had a dark violet color associated with wavelengths of 436 nm, 525 nm and 620 nm.

The authors observed that the separate biological treatment was efficient to remove COD but not the color in the residual water, additionally, the process lasted five days. On the contrary, when FMBR (24 h) and PMR (2 h) were an integrated system, removal was higher than 93% and 99% for color and COD, respectively. Our results were similar to the work reported by Deveci, however, we highlight that COD, BOD_5 and CU concentrations were higher in this investigation. Never the less, the complete sequence in the plant eliminated more than 95% of the three variables analyzed. Henceforth, demonstrating use of more than one ligninolytic fungus in association with aerobic heterotrophic bacteria could be an alternative treatment, substantially differing from conventional activated sludge reactors in which bacteria (up to 90%), protozoa, metazoans and microalgae predominate (Xia *et al*., 2018). On the other hand, use of combined processes makes it possible to decrease processing time and improve the quality of the final effluent that can be reused in other productive process (Blanco *et al*., 2018; Sathya *et al*., 2019).

Table 2. Performance of Each Unit which conform the pilot plant.

Units	**Time (h)**	**pH**	**COD mg/L**	**BOD5 mg/L**	**CU**
Equalization Tank (ET)	0.5	7	4033	2750	2010
Secondary Biological reactor (SBR)	24	6.9	1008	687	1103
Secondary Setted (SS)	2	5.4	705	481	678
Tertiary Photocatalytic Reactor (TPR)	6	5.8	105	72	50
Tertiary Settled (TS)	2	6	74	10	7
Filter Systems (FSAC)	2	6	50	8	3
Total Removal (%)	NA	NA	98	99	99

Conclusions

The results of this study at laboratory scale suggest that combination of biologic, physical and chemical processes, which are integrated through a pilot plant, are an option for the centralized treatment of synthetic wastewater, which is similar to that produced during teaching activities in Colombian university laboratories. The fungi - bacteria sludge assists in the elimination of high initial contaminant charge, whereas the photocatalytic process eliminates recalcitrant organic matter, obtaining removal percentages higher that 95% for CU, COD and BOD_5 at 37 h of process.

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Nomenclature

ANOVA:	Variance Analysis
FBS:	Bacterial Fungal Sludge
BOD_5:	Biochemical Oxygen Demand
CFU:	Colony Forming Units
COD:	Chemical Oxygen Demand
COOH:	Carboxyl
ET:	Equalization Tank
FSAC:	Filters of sand and activated Carbon
FS:	Fungal Sludge
HT:	Homogenization Tank
$-NH_3$:	Amino
OH:	Hydroxyl
pH:	Potential Hydrogen Ionic
SBR:	Secondary Biological Reactor
SEM:	Scanning Electron Microscopy
$-SO_3$:	Sulfonate
SS:	Secondary Settler
SST:	Total Solids Suspended
SSW:	Synthetic Wastewater
ST:	Tertiary Settler
TiO_2:	Titanium Dioxide
TPR:	Tertiary Photocatalytic Reactor
CU:	Color Units
UV:	Ultraviolet Light
W:	Watts
XRD:	X-ray Diffraction

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