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Articles

Assessment of the utilization rate of organic and nitrogenated substrates by the microorganisms in a sequencing batch reactor treating tannery wastewater

Evaluación de la tasa de utilización de sustratos orgánicos y nitrogenados por los microorganismos en un reactor discontinuo secuencial

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Abstract

In this article, the utilization rate of organic and nitrogenous substrates contained in tannery wastewater by a sequential batch reactor is evaluated. Two factorial experimental designs (FEDs) were implemented. FED1 (2^2) used granular biomass, the aeration sequence (oxic, anaerobic-oxic) and cycle duration (6 and 24 h). FED2 (4 x 3) included suspended biomass, reaction phases (anoxic I, oxic, anoxic II (CND: conventional nitrification-denitrification) and (SND: simultaneous nitrification-denitrification), filling time (fast, slow and in stages). The substrates examined were chemical oxygen demand (COD), ammonium (NH_4^+-N) and total nitrogen Kjeldahl (TKN). The utilization rates were COD (-100 and -200 mg l^{-1} h^{-1}) (FED1) and (-48 and -75 mg l^{-1} h^{-1}) (FED2), being 1.5 to 2 times higher in FED1 than FED2. In TKN (-5 mg l^{-1} h^{-1}), and NH_4^+-N (-30 mg l^{-1} h^{-1}), they were significant in the anoxic I and oxic phases.

Keywords: Sequencing batch reactor, substrate utilization rate, nitrification, denitrification.

Resumen

En este artículo se evalúa la tasa de utilización de sustratos orgánicos y nitrogenados contenidos en un agua residual de tenería por un reactor por carga secuencial. Se implementaron dos diseños experimentales factoriales (FEDs). El FED1 (2^2) usó biomasa granular, la secuencia de aireación (óxica, anaeróbica-óxica) y duración del ciclo (6 y 24 h). El FED2 (4 x 3) incluyó biomasa suspendida, fases de reacción (anóxica I, óxica, anóxica II (CND: nitrificación-desnitrificación convencional) y (SND: nitrificación-desnitrificación simultánea), tiempo de llenado (rápido, lento y por etapas). Los sustratos examinados fueron la demanda química de oxígeno (COD), amonio (NH_4^+-N) y nitrógeno total Kjeldahl (TKN). Las tasas de utilización resultaron COD (-100 y -200 mg l^{-1} h^{-1}) (FED1) y (-48 y -75 mg l^{-1} h^{-1}) (FED2), siendo de 1.5 a 2 veces mayor FED1 a FED2. En TKN (-5 mg l^{-1} h^{-1}), y NH_4^+-N (-30 mg l^{-1} h^{-1}), que fueron significativas en la fases anóxica I y óxica.

Palabras clave: reactor por carga secuencial, tasa de utilización del sustrato, nitrificación, desnitrificación.

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Introduction

In the tannery, the final product is the finished leather obtained from a process of transformation of the raw material (hides and salted skins), which consists of four stages (Stoop, 2003; Laurenti, Redwood, Puig, & Frostell, 2017): 1) beamhouse (curing and storage, soaking, trimming, dehairing and liming, deliming and batting); 2) tanyard (pickling, tanning, samming and splitting); 3) post-tanning (shaving, retanning, dyeing, fat liquoring, drying), and 4) finishing (mechanical finishing, coating).

The sequence of nine steps is an approach within the stages from the beamhouse to the finishing of the leather production system, which involves (Stoop, 2003): 1) soaking, adding water and additives to remove sodium chloride from the skins; 2) trimming, removing claws, ears and tails; 3) liming and dehairing, removing epidermis and hair by applying lime, sodium sulfide and enzymes; 4) fleshing, removing the subcutaneous layer manually or by machines; 5) deliming of the skin by adjusting the pH to 8 to neutralize most of the alkaline substances present in the skin (now called grains); 6) batting grains by means of pancreas enzymes; 7) pickling, partial hydrolysis of skin proteins and lipids. To obtain tanning conditions, the grains have to be handled with acid without swelling the skin. Therefore, they are treated with an aqueous solution of acids and salt. In addition, fungicides and bactericides are added, 8) tanning, the grains are saturated with a Cr^{3+}

solution, and 9) dehydration, some of the water is removed from the grains by manual or mechanical pressure.

The water footprint of tanneries whose process ranges from rawhide to finished leather is 130 to 170 lm^{-2} of processed leather (Laurenti *et al*., 2017). Leather tanning and processing generate wastewater whose main polluting chemicals are lime, sodium sulfide, ammonium salts, sulfuric acid, chromium salts and vegetable tanning materials (Sreeram & Ramasami, 2003), which require effective biological treatment before being discharged to receiving waters.

Among the biological treatment processes of suspended biomass used for the removal of the carbonaceous organic matter and nitrogenated compounds, sequencing batch reactor (SBR) is an activated sludge treatment system whose operation is based on the sequence of filling and emptying cycles.

From the 1970s to the present, SBR has been operated to treat domestic, industrial and synthetic wastewater. With regard to the influent substrate for SBR treatments, the raw domestic wastewater fed to SBR has varied in its composition, finding a COD ranging between 158 and 2 000 mg l^{-1}, NH_4^+-N of 10 to 185 mg/l (Irvine, Miller, & Bhamrah, 1979; Carucci, Chiavola, Majone, & Rolle, 1999; De-Silva y Rittmann, 2000; Palma-Costa & Manga, 2005; Su & Yu, 2005; Guo *et al*., 2009; Del-Rio *et al*., 2012; Kocijan & Hvala, 2013; Fernandes, Jungles, Hoffmann, Antonio, & Costa, 2013; Isanta *et al*., 2013; Ni, Joss, & Yuan, 2014).

The raw industrial wastewater provided to SBR by the tanneries has been fed in the following intervals, COD 845-5,584 mg l^{-1}, NH_4^+-N of 10 to 185 mg l^{-1} (Di-Iaconi, Lopez, Ramadori, Di-Pinto, & Passino, 2002; Freytez, Márquez, Pire, Guevara, & Pérez, 2019a; Freytez, Márquez, Pire, Guevara, & Pérez, 2019b; Freytez, Márquez, Pire, Guevara, & Pérez, 2020) pigs, COD of 400 mg l^{-1}, NH_4^+-N of 40 mg l^{-1} (Carrasquero *et al*., 2014), birds, COD 4,790 mg l^{-1}, NH_4-N of 274 mg l^{-1} (Alleman & Irvine, 1980).

The synthetic substrate fed to SBR has consisted mainly of sodium acetate combined with other chemical compounds, where COD ranged from 800 to 55 000 mg l^{-1}, NH_4^+-N from 30 to 10 000 mg l^{-1} (De-Kreuk, Heijnen, & Van Loosdrecht, 2005; Luo, Yang, Han, & Sun, 2014) as well as sucrose and other chemical compounds (Vázquez-Padin *et al*., 2010; Jungles *et al*., 2011).

According to a sample of 43 studies mentioned above, 67 % of these corresponded to SBR application with suspended biomass instead of granular biomass. COD and NH_4^+-N removal efficiency for suspended biomass ranged between 80 and 95 % for 72 % of studies, and between 40 and 80 % for 28 % of the studies. Regarding the efficiency of granular biomass, 40-80 % for 70 % of the studies, 80-90 % for the rest.

In this sample of studies, the sequential biological phases and cycle durations have been configured in 80 % of studies as oxic, 15 % of the studies in two sequential biological phases, anoxic-oxic, and 5 % in three sequential biological phases, anoxic-oxic-anoxic. The oxic phase duration was found being of 2 to 6 times greater than anoxic phase time.

In activated sludge system (ASS), the microorganisms carry out an aerobic metabolism obtaining the carbon and energy sources from chemical oxidation reactions of COD (chemoheterotrophs) and nitrogenated matter (nitrifying bacteria or chemoautotrophs).

In ASS, pure substrate experiments have hypothesized that municipal wastewater is composed of two fractions (Dold & Ekama, 1981): (a) a readily assimilable soluble fraction which is utilized at a very rapid rate, and (b) a slowly biodegradable particulate fraction which requires storage and enzymatic breakdown prior to transfer through the cell wall.

Since the 1940s, utilization of the stored substrate for synthesis has been modeled by a Monod-type relationship the concentration of the stored substrate with the specific growth rate of active organisms (Dold & Ekama, 1981).

The exact mechanism for utilization of two types of substrates by the microorganisms has not yet be stated established from extensive simulation studies. In this investigation, the novelties consist of estimating the substrate utilization rate of organic and nitrogenated substrates by the microorganisms in a SBR operated under single (oxic) and combination of two (anaerobic-oxic) and three (anoxic I-oxic-anoxic II) sequential biological phases. The substrates are made up by COD, NH_4^+-N and TKN. The substrate utilization rate (SUR) is estimated under conditions: a) oxic for a cycle duration time of 24 h; b) oxic for a cycle duration time of 6 h; c) oxic-anaerobic for a cycle duration time of 24 h; d) oxic-anaerobic for a cycle duration time of 6 h, and e) anoxic-oxic-

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anoxic for a cycle duration time of 12 h. The observed trends will provide the dynamic of substrate utilization rate in the sequential biological phases, allowing to determine the microorganism performance to transform the substrate following different cycle durations, biomass types, conventional and simultaneous nitrification-denitrification processes and biological sequences in SBR. The specific objectives are the followings: 1) characterization of organic and nitrogenated compounds in the tannery wastewater; 2) estimation of utilization rate of organic and nitrogenated substrates; 3) assessment of utilization rate of organic and nitrogenated substrates.

Methods

The applied method included the following six stages: 1) construction of SBR; 2) selection of the substrate; 3) acclimatization of the biomass; 4) experimental design; 5) statistical analysis of results, and 6) evaluation of the performance of SBR (Figure 1).

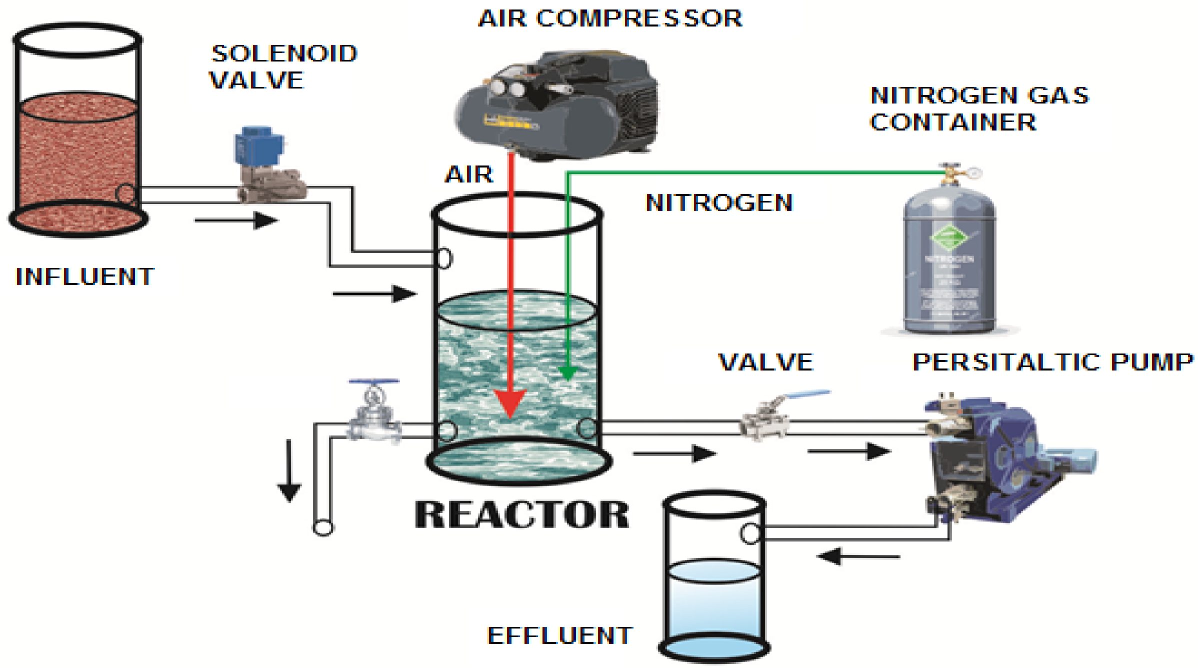

Figure 1. Scheme of SBR treatment system. Source: Own elaboration.

Construction of the SBR

Reaction system for experimental treatments in one and two biological phases was composed of the following components: a cylindrical container 50 cm high by 10 cm in diameter, with a capacity of 3 liters and a useful volume of 2 liters, constructed from transparent acrylic material based on polymethyl methacrylate (Freytez *et al*., 2019c). The reactor had one point for the inlet of wastewater and two points for drainage. The inlet was located in the upper part at 34 cm from the bottom where the wastewater is fed and two outlets at the bottom. The first outlet was located 8 cm from the bottom where the treated effluent is discharged and the second outlet is at the bottom of the reactor and is used as a drain to clean the system.

Digital timers (Exceline, Venezuela) were used to control in an automated way the operating reactor. These devices guaranteed the activation and deactivation of the electronic components used during the treatment of tannery wastewater. A solenoid valve (ASCO, USA) of 0.6 cm diameter was installed to manage the filling of the reactor. For the effluent discharge from the reactor, a peristaltic pump (Easy Load II, Masterflex L/S, Cole Parmer, USA) led the discharge after application of the treatment (Freytez *et al*., 2019a; Freytez *et al*., 2019b; Freytez *et al*., 2019c) (Figure 1).

Selection of the substrate

The main physicochemical characteristics of the effluent from a tannery industry associated to the substrate included the following parameters: 1) pH between 9 and 9.56; 2) COD: 4 904 and 6 264 mg l^{-1}, biochemical oxygen demand (BOD): 1 347 and 6 264 mg l^{-1}, TKN: 221 and 299 mg.l^{-1}, NH_4^+-N: 2 mg l^{-1}, chloride: 58 703 and 58 904 mg l^{-1-1} and chromium: 3 mg l^{-1} (Freytez *et al*., 2019a; Freytez *et al*., 2019b; Freytez *et al*., 2019c). The values of the variables were measured to the effluents of the processes of furring, tanning, dyeing, tanning-dyeing and wastewater stored in the lagoon and determined in the laboratory following the standardized methods for the analysis of drinking water and wastewater (APHA-AWWA-WPCF, 2005). The tannery from which the wastewater was obtained for both studies was located, Lara State, Venezuela.

Acclimatization of the biomass to the substrate

In the FED-1, before starting the reinforced acclimatization process of the granular biomass, a preliminary stage was developed during which, three parameters were controlled in order to verify the biomass performance in the COD removal, by feeding SBR with a synthetic water (Freytez *et al*., 2015). The SBR was fed with 2 lof synthetic water and 25 g.l^{-1} of granular biomass. The synthetic water was composed by the following chemical substances (Freytez *et al*., 2015), sodium acetate, $NaCH_3COO$ (4.5 g.l^{-1}), ammonium chloride, NH_4Cl (0.25 g.l^{-1}), dipotassium phosphate, K_2HPO_4 (0.045 g.l^{-1}), calcium carbonate, $CaCO_3$ (0.03 g.l^{-1}), magnesium sulfate heptahydrate, $MgSO_4.7H_2O$ (0.025 g.l-1), and ferrous sulfate heptahydrate, $FeSO_4.7H_2O$ (0.02 g.l^{-1}). The same components for the synthetic water to evaluate the granular biomass performance in the removal of organic and nitrogenated substrates, varying in an interval for COD (200-500 mg.l^{-1}) and NH_4^+-N (20-100 mg.l^{-1}) by SBR were used by Luo *et al*. (2014) and Vázquez-Padin *et al*. (2010) finding high removal efficiencies (84-98 %).

Two types of biomass were employed to remove organic and nitrogenated substrates in SBR, granular and suspended. The granular biomass was obtained from a laboratory-scale biological reactor that processed synthetic effluents with characteristics similar to those of the tannery. A natural process of selection of the existing microorganisms was found, through the work cycles of the system, this favored the growth and establishment of the floc-forming microorganisms, thus eliminating those filamentous bacteria that could generate delays in sedimentation times (Freytez *et al*., 2019a). The granular biomass was acclimatized for the organic substrate measured as COD during a time period of 60 days. The Archimedes Principle was applied for the characterization of the granular biomass at the beginning of the acclimatization process, which was carried out by the determination of the biomass density, whose average was of 1.19 g ml^{-1} and standard deviation of 0.13 g ml^{-1}. The mean size of the granules in the biomass varied from 1-5 mm, for which 100 granules were taken and their diameters were measured with a graduate instrument (Freytez *et al*., 2019c). The microbial count in the stage of acclimatization of the granular biomass is shown in Figure 2 during six stages in which the dilution of tannery raw wastewater was varied. The results of the stages 1 and 2 gave a microbial count in the plates without dilution and in the serial dilutions of 10^{-1} to 10^{-3} CFU (colony-forming unit) ml^{-1}. During stages 3, 4 and 5, the count of viable microorganisms had a slight decrease as the dilution was diminished (Figure 2). The results found in those stages were 6.3 x 10^5 CFU ml^{-1} (Stage 3), 2.5 x 10^5 CFU ml^{-1} (Stage 4) and 1.3 x 10^5 CFU ml^{-1}. For stage 6, the microorganism count

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decreased to 4 x 10^3 CFU ml^{-1}. A significant growth was observed that was decreasing in proportion to the dilution, confirming the presence of microorganisms in the environment that were responsible for the removal of organic matter, measured in the form of COD.

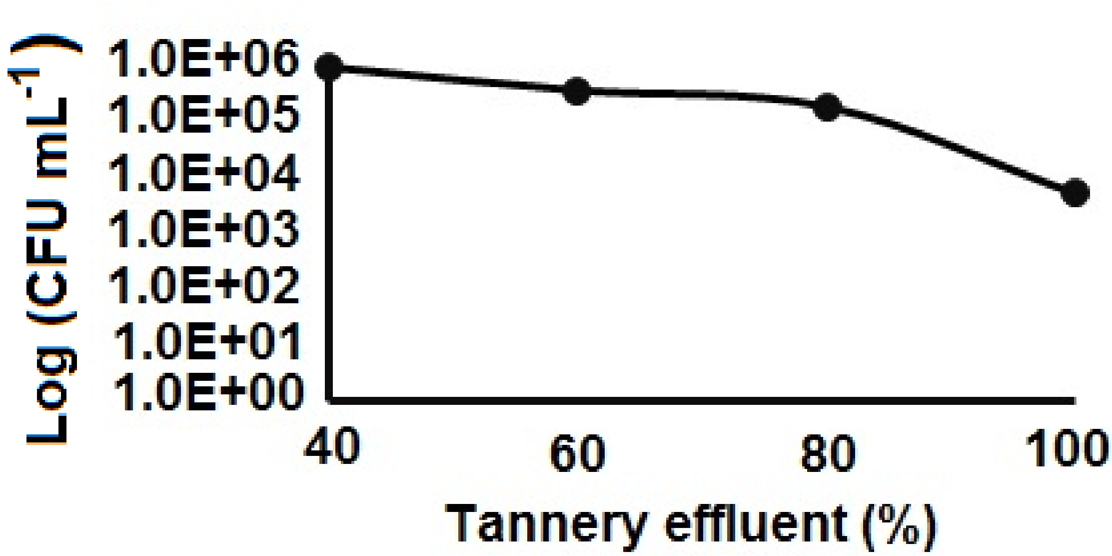

Figure 2. Microbial count integrating the granular biomass during the acclimatization stage. Source: Own elaboration.

The suspended biomass was extracted from biological reactors of activated sludge operating for tannery. The reactor was fed with 2lof wastewater (30 % biomass, 70 % effluent), while the suspended biomass was acclimated to the characteristics of the effluent. The acclimatization time of the granular biomass for the organic substrate measured as COD was 60 days. The dilution of raw tannery wastewater was decreased obtaining a COD in the influent to SBR, which was increased from 2 000 to 4 000 mg l^{-1}. As well as, the COD in the effluent to SBR varied between 200 and 2 000 mg l^{-1} (Figure 3). The COD removal resulted ranging between 60 and 80 % (Figure 3). The establishment of a population of microorganisms able to develop their metabolic processes was achieved despite the inhibitory compounds and scarce biodegradable organic matter present in the tannery effluents.

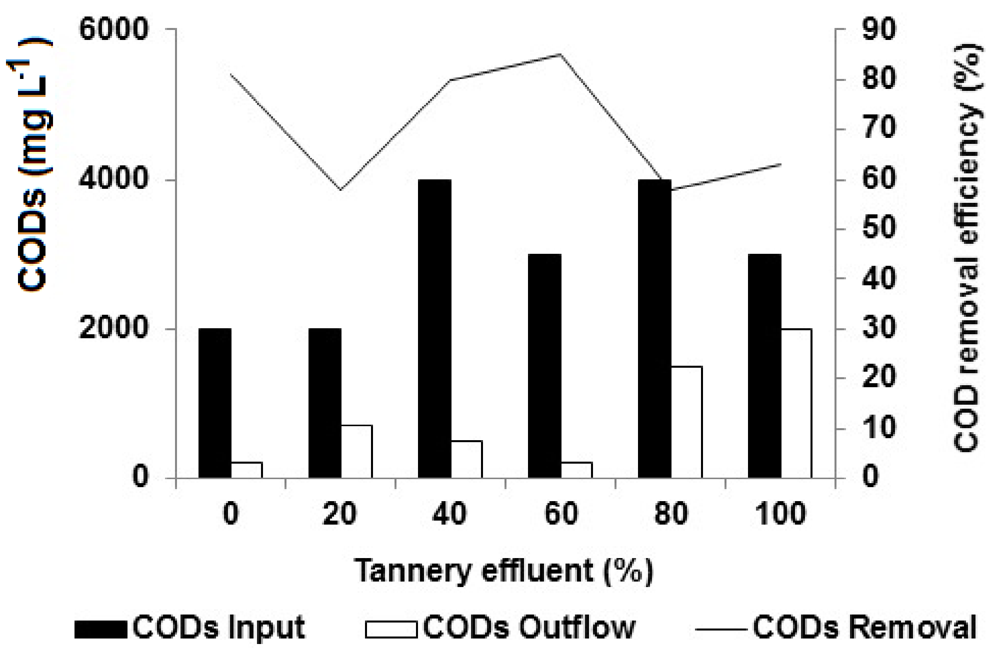

Figure 3. Variation of COD during the acclimatization stage of the suspended biomass. Source: Own elaboration.

Experimental design

Two factorial experimental designs (FED) were implemented. At FED1, SBR was tested to remove organic substrate (COD) with granular biomass. The FED1 was a regular two-factor design 2^2, for four treatments (T1 to T4). The experimental factors were the cycle duration fixed in two levels (6 and 24 h), as well as the sequential biological phases, which consisted of two types of aeration sequences (oxic and anaerobic-oxic).

The FED2 was made up of two experimental factors adjusted in four and three levels (4x3). In the FED2, the suspended biomass was performed to remove organic and nitrogenated substrates contained in the tannery wastewater. The factorial arrangement led to 12 treatments. The first factor was the filling time with three levels, fast, slow and by stage. The second factor was the sequential biological phases with four levels: 1) anoxic I; 2) oxic; 3) anoxic II–simultaneous nitrification-denitrification (SND), and 4) Anoxic II conventional nitrification-denitrification (CND). The responses or dependent variables in the experiments were three (COD, TKN and NH_4^+-N).

The experimental factors such as cycle duration and filling time in the ED-1 and ED-2 were configured based on a review of 43 scientific studies carried out in the period 1979-2017 (Freytez & Márquez, 2021). With regard to the cycle duration, it was fixed 6, 12, and 24 h. Based on a sample of 42 laboratory-scale SBRs reactor, it was found that the cycle durations of 12 and 24 h were included within the low test frequencies of 6 and 5, respectively, making them distinctive (Freytez *et al*., 2019a). With respect to filling time, this varied in fast 0.083 h, slow 1 h and in stages 0.333, 0.25, 0.25 and 0.166 h, finding a constant temporal trend. Based on a sample of 42 studies of laboratory-scale SBRs, it was found that the filling time was adjusted in fast, slow and by stages were within the interval of (μ ± 1σ), being included within the high frequency of tests (Freytez *et al*., 2019b).

Procedure of experimental treatments

The experimental treatments tested in the SBR operation associated with FED included sequences of biological processes from one to three phases. The biological processes were oxic (FED1), anaerobic-oxic (FED1) and anoxic-oxic-anoxic (FED2). Figure 4 shows the time settings for four stages (filling, reaction, settling and drain) within the SBR operation from Treatment 1 (T1) to Treatment 5 (T5):

a) **Treatment 1 (T1)-FED1**: COD removal was carried out in a single biological phase corresponding to the oxic developed for a cycle duration of 24 h (Figure 4a): T1 was carried out during 24 h of operation cycle, which was distributed 3 minutes for filling time, 1 430 minutes of reaction time, 2 minutes for settling time and 5 minutes for withdrawal time. T1 was composed by the four stages as follows (Freytez *et al*., 2019c):

a1) Filling stage: The substrate was fed to the reactor by gravity flow from the constant charge container (Figure 1). A solenoid valve (ASCO, USA) with a diameter of 0.6 cm was used to control the feed rate and achieve a 3 min. anoxic fill period (Figure 1).

a2) Reaction stage (oxic): Air was introduced through a fine bubble diffuser at the bottom of the reactors (2.5 $lmin^{-1}$). Aeration was supplied through an air compressor (Elite 801, Hagen Inc., China) (Figure 1). The dissolved oxygen concentration was measured as percentage of the saturation concentration (8 mg l^{-1}). The SBR was aerated providing constant volume air by adjusting the stirrer and aerator in position for a time of 1 430 minutes.

a3) Settling stage: This phase allowed the separation of solids to obtain a clarified supernatant as an effluent. The SBR operated at constant volume by adjusting the motor and agitator in the off position for 2 minutes.

a4) Draining stage: In this phase, the clarified water was extracted from the reactor. The SBR was operating to minimum volume adjusting the motor and stirrer in off position by 5 minutes.

b) **Treatment 2 (T2)-FED1**: The COD removal was executed for a cycle duration of 6 h, including an oxic phase of reaction (Figure 4b): T2 was developed with the same SBR operating sequence as T1, changing the cycle duration from 24 h to 6 h. The stages had a duration as follows:

b1) Filling stage: 3 minutes.

b2) Reaction stage (oxic phase): The aeration was calibrated to maintain a dissolved oxygen concentration of around 8 mg l^{-1} in the bioreactor during the aeration cycle of 350 minutes.

b3) Settling stage: 2 minutes.

b4) Draining stage: 5 minutes.

c) **Treatment 3 (T3)-FED1**: COD removal occurred for a cycle duration of 24 h. The reaction stage consisted of two sequential biological phases, Anaerobic-Oxic (Figure 4c):

c1) Filling stage: The filling time was adjusted in 3 min. According to Freytez *et al*. (Freytez *et al*. 2019a; Freytez *et al*. 2019b) the COD reached a decreasing around 75 % for soluble substrates, while it varied close to 30 % for particulate substrates during the anoxic filling time.

c2) Reaction stage: The reaction phase included two biological processes (anaerobic-oxic).

- Reaction phase (anaerobic phase): The SBR was fed with N_2 gas by intermittent sparging to displace the oxygen gas molecules for a period of 28 minutes to keep the solids in suspension and the feed under anaerobic conditions. After that time, the development of the anaerobic phase lasted about 331 minutes (Figure 4). The SBR was operated at constant volume by setting the agitator to the on position and the aerator to the off position.

- Reaction phase (oxic phase): Aeration intensity was maintained at 2.5 l h^{-1} generating a dissolved oxygen concentration varying between 2 and 8 mg l^{-1} during 1 070 minutes (Figure 4).

c3) Settling stage: 2 minutes (Figure 4).

c4) Draining stage: 5 minutes (Figure 4).

d) **Treatment 4 (T4)-FED1**: COD removal occurred for a cycle duration of 6 h including two sequential biological phases, anaerobic-oxic (Figure 4d):

d1) Filling stage: This time corresponded to 5 minutes being added to the first anoxic stage.

d2) Reaction stage: The reaction phase included three biological processes, which are anaerobic-oxic.

- Reaction stage (anaerobic phase): In this phase, SBR operation was to constant volume, adjusting stirrer in on position and aerator in off position during a period of 225 minutes.

- Reaction stage (oxic phase): The dissolved oxygen was supplied from the bottom of the reactor by using air spargers during 315 minutes.

d3) Settling stage: The settling occurred during 45 minutes.

d4) Draining stage: A peristaltic pump was used to discharge the effluent (at medium height in the column reactor) during 15 minutes.

e) **Treatment 5 (T5)-FED2**: For T5, the FED2 employed suspended biomass for the tannery wastewater treatment, which was applied under the following three experimental factors*:* 1) filling: Three filling times were adjusted (slow, rapid and by stages); 2) Cycle duration was fixed in 12 h, and 3) three sequential biological phases were applied to the SBR (anoxic I-oxic-anoxic II). T5 was integrated by four stages (Figure 4e):

e1) Filling stage: The objective of this phase was the addition of substrate to the reactor. The reactor was filled with the tannery effluent to the maximum volume adjusting the stirrer and aerator in off position, the phase duration was adjusted in three filling times (slow, rapid and by stages).

e2) Reaction stage: The reaction phase included three biological processes, which are anoxic-oxic-anoxic.

- First anoxic phase: In this phase, a preliminary denitrification was carried out by which the nitrogen of the nitrates contained in the wastewater is transformed biologically in nitrogen gas in the absence of oxygen, being the phase duration of 225 minutes (Figure 4e). The simultaneous nitrification-denitrification process occurred.

- Aeration phase (oxic): In this phase occurred the biological transformation of organic matter from wastewater in cell tissue and various gaseous products. In the conversion, the Nitrosomonas oxidized the ammonium to nitrite, intermediate product, while the Nitrobacter transformed the nitrite into nitrate. The simultaneous nitrification-denitrification process occurred. The aerobic conditions were achieved by supplying air through a fine bubble diffuser placed at the bottom of the reactor connected to a compressor of type Elite 801 (Hagen Inc., China) of 0.21 kg cm^{-2}, 2.5 Watt h^{-1} and flow of 2.5 l min^{-1}, maintaining a minimum oxygen concentration of 2 mg l^{-1} in the system the phase duration was of 315 min (Figure 4e).

- Second anoxic phase: In this phase, the nitrification-denitrification process was carried out into SBR. The phase duration was 120 minutes (Figure 4e).

e3) Settling stage: The purpose of this phase was to allow the separation of the solids to obtain a clarified supernatant as effluent. The reactor was operating with stirrer and aerator in off position (Figure 1), the phase duration was 45 minutes (Figure 4e).

e4) Drainage stage: In this phase occurred the extraction of clarified water from the reactor. The reactor was operating with stirrer and aerator in off position (Figure 1), the phase duration was of 15 minutes (Figure 4e).

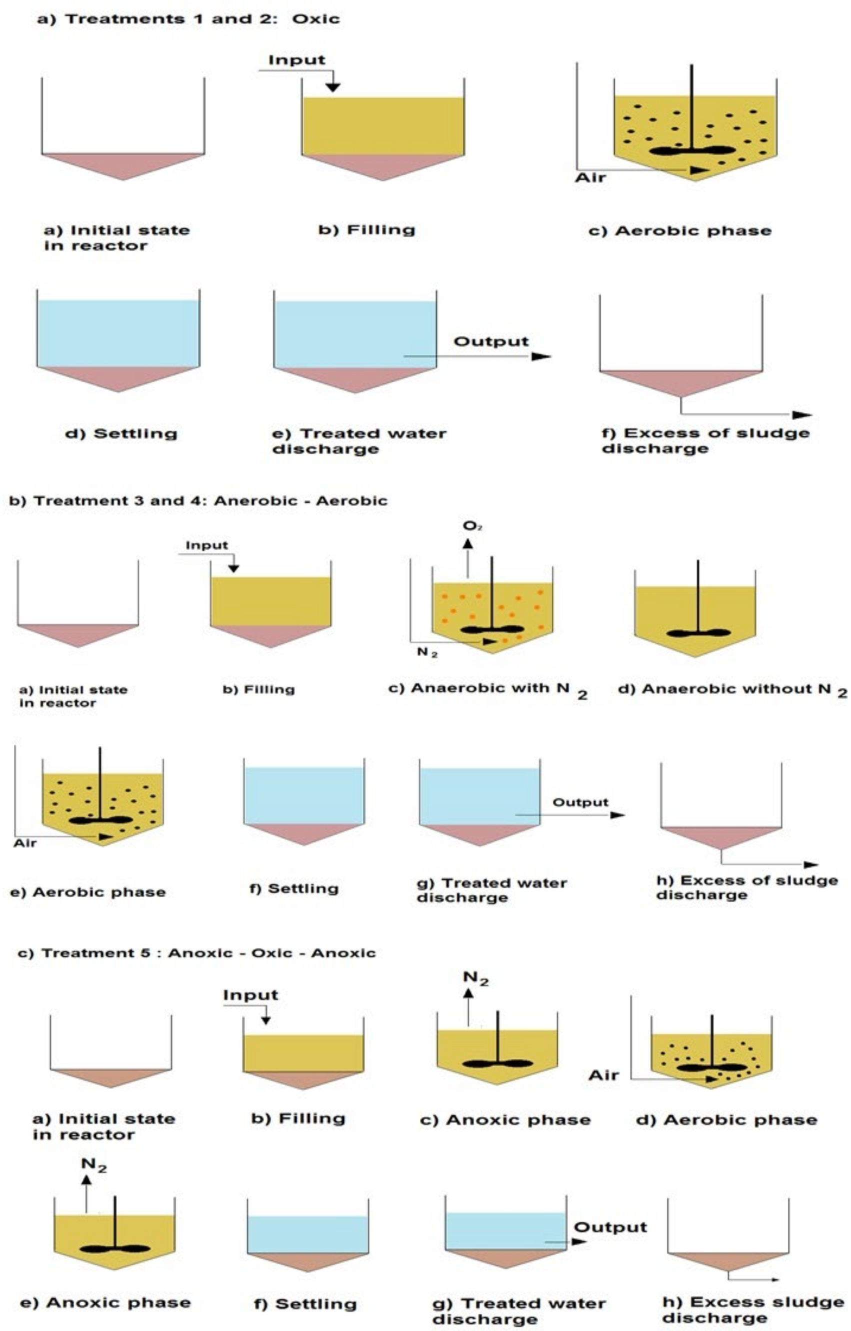

Figure 4. Treatment phases of sequencing batch reactor: a) treatment 1 and 2: Oxic; b) treatment 3 and 4: Anaerobic-oxic; c) treatment 5: Anoxic-oxic-anoxic. Source: Own elaboration.

Statistical analysis of results

Statistical analysis of the results was made using the graphs of mean values, which are applied as an instrument to represent the variation of COD, NH_4^+-N, and TKN in the inlet-outlet of five SBR treatments. The graphs of mean values summarize a data sample through three statistics: 1. Lower limit, 2. Mean, 3. Upper limit (Spiegel & Stephens, 2009). The analyst can determine which means are significantly different from which others using the Fisher's least significant difference procedure by looking at whether or not a pair of intervals overlap in the vertical direction. A pair of intervals that do not overlaps indicates a statistically difference between the means at a determined confidence level (Spiegel & Stephens, 2009).

Evaluation of SBR performance

Evaluation of SBR performance was obtained by graphically comparing the substrate utilization rate (dS/dt) from five treatments.

Results

The results of the comparison of control variables in SBR treatments for granular biomass, T1 to T4, and suspended biomass, T5, are shown in Figure 5, where the control variables have been represented using graphs of mean values.

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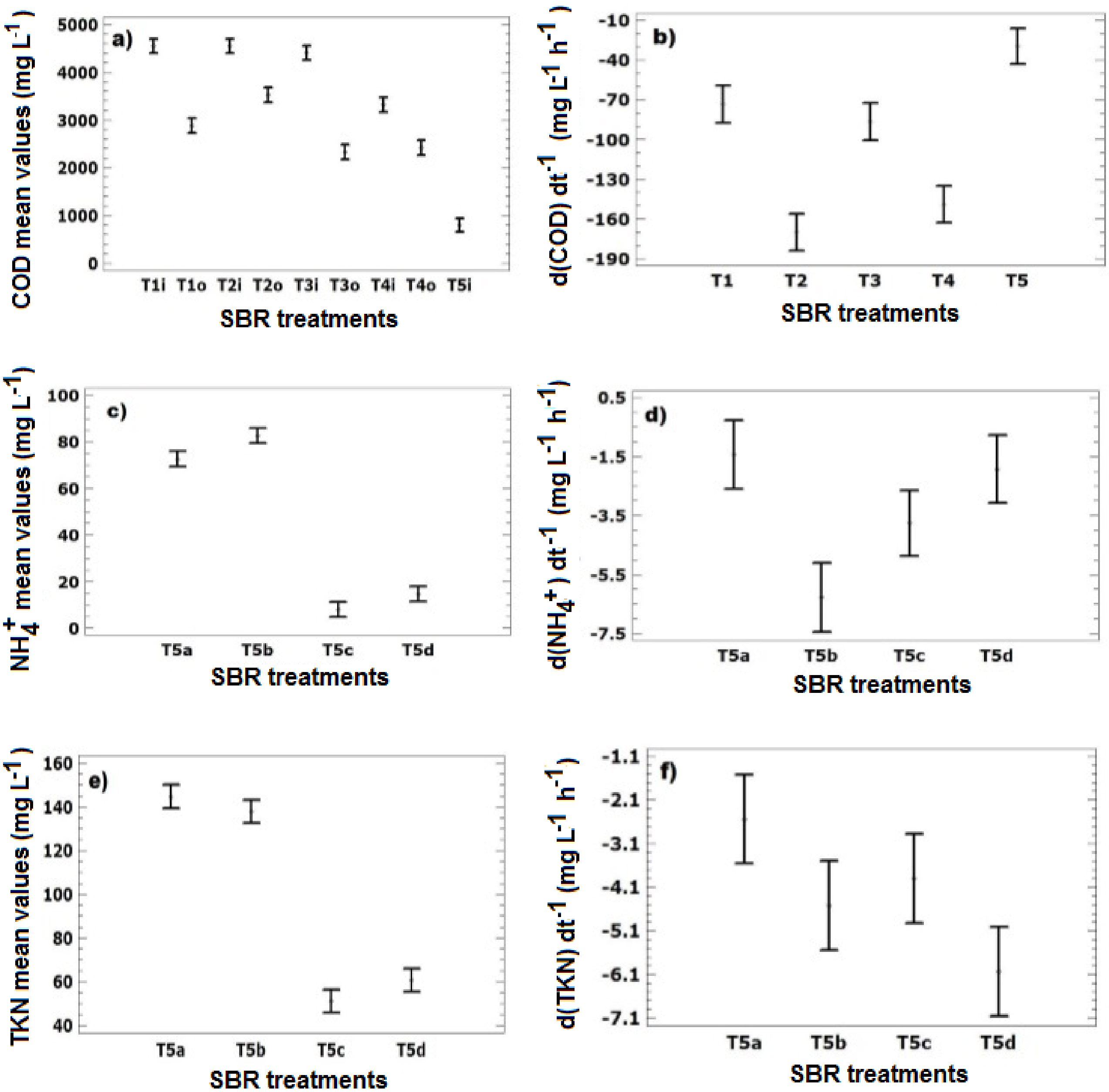

Figure 5. Comparison of control variables of SBR treatments: a) COD *versus* SBR for treatments T1 to T4 in SBR; b) $dCOD/dt$ *versus* for treatments T1 to T4 in SBR; c) COD for treatment T5 in SBR; d) $dCOD/dt$ for treatments T5 in SBR; e) NH_4^+-N for treatment T5 in SBR;

f) $dNH_4^+ - \mathrm{N}\,/dt$ for treatment T5 in SBR; g) TKN for treatment T5 in SBR; h) $dTKN/dt$ for treatment T5 in SBR. T1: Oxic for a cycle duration of 24 h. T2: Oxic for a cycle duration of 6 h. T3: Anaerobic-oxic for a cycle duration of 24 h. T4: Anaerobic-oxic for a cycle duration of 6 h. T5a: Anoxic I for a cycle duration of 12 h. T5b: Aeration for a cycle duration of 12 h. T5c: Conventional nitrification-denitrification (Anoxic II) for a cycle duration of 12 h. T5d: Simultaneous nitrification-denitrification (Anoxic II) for a cycle duration of 12 h. Source: Own elaboration.

COD removal using granular biomass

The influent COD values for T1 to T4 varied between 2 916.7 mg l^{-1} and 6 666.7 mg l^{-1} (Figure 5a, Table 1), being 1.17 to 2.37 times greater than the effluent COD values for the SBR operation. For the T1 and T2 (Figure 5a, Table 1), the COD effluent diminished as the time was increased from 6 to 24 h. The microorganisms require 12 h additional for increasing the oxidation of COD values around 30 % with respect to T3 and T4. For T3 and T4 (Figure 5a, Table 1), the effluent COD values for cycle duration of 6 and 24 h were between 40 % and 90 % lower in the first with respect to the second. It was found that COD removal was increased between 30-40 % as the number of biological phases was increased within the SBR operating.

Table 1. Statistical parameters of organics substrates obtained in the factorial experimental design N°1 (FED1) and substrate utilization rate (SUR).

	t	COD	Mean	SD	Substrate utilization rate (SUR)	RE
	h	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1} h^{-1}	%
T1	0	CODt	5 873.0	376.2		
	24	CODt	3 311.6	230.6	-106.7	43.6
T2	0	CODt	5 873.0	376.2		
	6	CODt	4 112.8	306.9	-293.3	29.9
T3	0	CODt	5 485.7	161.9		
	24	CODt	2 828.5	170.4	-110.7	48.4
T4	0	CODt	4 090.8	513.2		
	6	CODt	2 919.2	937.2	-195.2	28.6

CODt: Total COD; SD: Standard deviation; SUR: Substrate utilization rate; RE: Removal efficiency.

Source: Own elaboration.

COD utilization rate by granular biomass

COD rate utilization ($dCOD/dt$) by the granular biomass is shown in Figure 5b. The graphs of mean values show that there is not statistically

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significant difference between the $dCOD/dt$ estimated in the SBR for the treatments from T1 a T4. However, the COD removal rate tends to be steady in T1 and T3 taking mean values of -100 mg l^{-1} h^{-1} associated to a cycle duration of 24 h. With respect to T2 and T4, COD removal rate shows a major variation occurring values of -350 to -400 mg l^{-1} h^{-1}, which represents three standard deviations (Figure 2b). Furthermore, the values that occur between 25 and 75 % time comprise an interval of -160 to -240 mg l^{-1} h^{-1}. This randomness could be associated due to influence of adaptation by facultative bacteria to the biological phase change in the multiple biological phases in a short period of time, the affinity by the substrate in some bacterial strains, the microbial behavior related to the exponential growth stage (Metcalf & Eddy, 1995).

For different data groups in SBR operating under T1 and T2, the kinetic parameters have demonstrated that the granular biomass can predominantly produce accumulation of COD concentration within the reactor. The mean value of the substrate accumulation rate ($r_{(m,S)}$) reached 92.27 mg COD l^{-1} h^{-1} for T1, and 117.85 mg COD l^{-1} h^{-1} for T2 (Freytez *et al*., 2020). With another data group, it was found a substrate utilization rate suggesting that the biodegradation occurred in a magnitude of -135.65 mg COD l^{-1} h^{-1} for T1, and -159 mg COD l^{-1} h-1 for T2 (Freytez & Márquez, 2021). This kind of biomass behavior can occur due to the toxic concentrations of the compounds to be degraded such as the chlorides in the raw wastewater of the tannery (58 804 mg l^{-1}) (Freytez *et al*., 2019a). High concentrations of some chemicals can cause major physical disruption of bacteria (*e.g.*, dissolution of membranes by solvents), or create competitive binding of a particular enzyme. In these cases, physical processes such as dilution, sorption, precipitation and volatilization, become important mechanisms (Guevara, 2016). In this study, it was demonstrated that dilution was an option to allow the COD removal by the biomass within the SBR with operating efficiencies varying between 70 and 80 % for a percentage in the tannery mixed wastewater/synthetic water from 20 to 60 % (Figure 5) (Freytez *et al*., 2019c; Freytez & Márquez, 2021).

The mean value of $r_{(m,S)}$ for T3-T4 (-56.76 mg l^{-1} h^{-1}) was close to the value found for T1-T2, meaning that there was a similar behavior of the granular biomass in the oxic and anaerobic-oxic phases with respect to the biodegradation of the substrate that composes the raw wastewater of tannery. As it was observed in acclimatization period, the colony forming units and COD removal were decreasing when the raw wastewater replaced to the synthetic water (Figure 2, Figure 3). This result is a proof that it is necessary to add a primary substrate to achieve a high microbial concentrations inducing a cometabolisms that lead to improve the COD removal within SBR operation (Guevara, 2016).

COD removal using suspended biomass

The COD removal in the three sequential biological phases, anoxic I -oxic- anoxic II using suspended biomass can be seen in Figure 5c and Table 2. COD is reduced in the anoxic I phase, T5a, due to the possible occurrence of anaerobic ammonium oxidation (anammox). In the oxic phase (T5b), COD increases slightly because of the nitrification process, transforming NH_4^+-N to NOx. In the second anoxic phase (T5c), COD shows steady state influenced by possible inhibitory and recalcitrant effects of chemical compounds such as heavy metals (chromium) and metal halides (chlorides) detected in the raw wastewater characterization (27, 28). The $(COD_i/COD_e)_{Ti}$ ratio in the first anoxic phase ranged in the order of 1.53-2.37 and in the remaining stage, it is less than unity, indicating that there is a possible COD accumulation (Table 2). When comparing T5 with respect to T1 to T4, it has been found that there is a similar relationship $(COD_i/COD_e)_{Ti}$ ratio estimated in the biological phases.

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Table 2. Statistical parameters of organic and nitrogenated substrates obtained in the factorial experimental design N° 2 (FED2), and substrate utilization rate

	t	**Filling**	**Variable**	**Mean**	**SD**	**Substrate utilization rate (SUR)**	**RE**
	h	**Time**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1}**	**mg l^{-1} h^{-1}**	**%**
T5	0	Rapid	CODt	1 485.8	84.6		
	0	Slow	CODt	1 174.8	127.0		
	0	By stage	CODt	1 353.9	105.7		
	12	Rapid	CODt	725.7	127.0	-63.3	51.1
	12	Slow	CODt	617.9	59.0	-46.4	47.3
	12	By stage	CODt	712	48.8	-53.4	47.4
T5	0	Rapid	NH_4^+-N	84	7.0		
	0	Slow	NH_4^+-N	70	4.0		
	0	By stage	NH_4^+-N	63.8	1.7		
	12	Rapid	NH_4^+-N	7.4	3.6	-6.3	91.1
	12	Slow	NH_4^+-N	20.3	4.7	-4.1	71
	12	By stage	NH_4^+-N	16.2	1.4	-3.96	74.5
T5	0	Rapid	TKN	164.5	12.1		
	0	Slow	TKN	141.7	6.7		
	0	By stage	TKN	127.7	3.5		
	12	Rapid	TKN	54.7	9.5	-9.1	66.7
	12	Slow	TKN	64.1	7.9	-6.4	54.7
	12	By stage	TKN	63.0	1.8	-5.3	50.6

CODt: Total COD; SD: Standard deviation; SUR: Substrate utilization rate; RE: Removal efficiency.

Source: Own elaboration.

COD utilization rate by suspended biomass

COD utilization rate by suspended biomass reaches a mean value of -40 mg l^{-1} h^{-1} in the first anoxic stage, T5a (Figure 5d, Table 2), confirming that the COD removal is occurring with a variation reaching to -100 mg l^{-1} h^{-1} for rate value with three standard deviation. In the remaining biological phases, T5b-T5d, COD utilization rate is steady and close to zero (Figure 5d), because of the occurrence of possible inhibitory effects on microbial metabolism.

Nitrogen removal by the suspended biomass

Ammonium removal by the suspended biomass

In the SBR operation of three biological sequential phases, ammonium removal is carried out between the first and second biological phase (Figure 5e, Table 2). In the first anoxic phase, the simultaneous process of anaerobic ammonium oxidation (anammox) is occurring due to the nitrification of the NH_4^+-N. Ammonium removal shows a decrease from 35 to 15 mg l^{-1}. In the oxic phase, the reduction of NH_4-N occurs from 45 to 15 mg l^{-1}. In the second anoxic stage, both CND (T5c) and SND (T5d), the ammonium removal is steady, decreasing from 25 to 15 mg l^{-1}.

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Ammonium utilization rate by suspended biomass

In the SBR operation of three biological sequential phases, in the first anoxic stage, the ammonium removal rate (dNH_4^+/dt) occurs between -1 and -10 mg l^{-1} h^{-1} (Figure 5f, Table 2). In the oxic phase, the ammonium removal rate is increased in a significant way due to the nitrification process, taking values between -1 and -30 mg l^{-1} h^{-1}. In the remaining anoxic phase under both SND and CND, the ammonium removal rate is stabilized in -1 to -10 mg l^{-1}.

Total Kjeldahl nitrogen (TKN) removal by the suspended biomass

In the SBR operation of three biological sequential phases, TKN is slightly reduced from 60 to 35 mg l^{-1} (Figure 5g, Table 2). In the first anoxic stage, the production of NOx by nitrification could contribute to limit the TKN removal, due to the possible simultaneous process of nitrification-denitrification. In the aeration stage, TKN diminishes in less proportion with respect to NH_4^+-N, which could be occurring because of the accumulation of NOx in the mixed liqueur in the reactor. The decrease in TKN could be associated with the oxidation of NH_4^+-N. In the second anoxic stage, inhibitory substances can influence the stabilization of the nitrogen forms.

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Total Kjeldahl nitrogen (TKN) utilization rate by the suspended biomass

In the SBR operation of three biological sequential phases (Figure 5h, Table 2), the TKN utilization rate $(dTKN/dt)$, shows a constant trend taking values between -5 and 5 mg l^{-1} h^{-1}, occurring reduction of NH_4^+-N and increase of NOx.

In general, it was found a pattern in the six conditions examined to evaluate the COD removal and nitrogenated compounds as a function of the SBR operating conditions with suspended biomass. Although the removal of the organic and nitrogenated substrates contained in the tannery wastewater was carried out under the influence of the inhibitory and recalcitrant substrates, it was considered that the effects were overcome due to the following aspects (Álvarez & Guevara, 2003; Guevara, 2016):

a) Most of the structural characteristics of the organic and nitrogenous compounds present in the tannery wastewater could be within the central metabolic pathways recognized by the degrading enzymes produced by the microorganisms.

b) Primary substrate concentration was present in a suitable amount to sustain high microbial concentrations in those phases of the SBR operating, where the removal of the substrates was significant.

Discussion

Treatments using granular biomass

COD removal in SBR using granular biomass

Based on the results of experimental design No. 1 shown in Figure 5 and Table 1, the following hypotheses and findings are suggested:

- In treatments using the individual oxic aeration sequence for tannery wastewater in the SBR (T1 and T2), the average COD removal efficiencies were 48.4 % (T1) and 28.6 % (T2) under oxic SBR operating conditions (Table 1). Some of the factors to be introduced to improve the treatment would be: Increase in the duration of the cycle, increase in the variety of autotrophic and heterotrophic microbial strains until forming a network of microbial consortium that contribute through commensalism to cometabolically degrade the substrate, dilution of the substrate in the influent to the SBR, inclusion of organisms whose excreted enzymes act on the organic complexes on an extracellular scale (Guevara, 2016).
- In the treatments using the anaerobic-oxic aeration sequence (T3 and T4), it is found that the average COD removal efficiencies were

42.51 % (T3) and 26.89 % (T4) (Figure 5, Table 1). The increase in the performance in the removal of organic substrates (COD) from tannery wastewater would be achieved by stimulating the growth of heterotrophic biomass (bio-stimulation) by introducing macro and micronutrients to achieve the fermentation of organic substrates in the anaerobic stage, as well as an increase in the duration of the cycle to at least 24 h, as a significant factor to improve the biodegradation of tannery wastewater (Guevara, 2016). By comparing the COD results in terms of influent and removal efficiencies with other studies (Table 3, Table 4), it was found that for CODs lower than 10^3 mg l^{-1} and cycle duration between 8-12 h, the removal efficiencies were upper than 90 % (Irvine *et al.*, 1979; Alleman & Irvine, 1980). For CODs upper than 10^3 mg l^{-1} and cycle duration ranging between 12 and 14 h, COD removal resulted to vary from 60 to 97 % (Lefevbre *et al.*, 2004; Ganesh, Balaji, & Ramanujam, 2006; Murat, Insel, Artan, & Orhon, 2006; Pire-Sierra, Palmero, Araujo, & Díaz, 2010; Freytez *et al.*, 2015; Freytez *et al.*, 2019a; Freytez *et al.*, 2019b; Freytez *et al.*, 2019c; Freytez, Márquez, Pire, Guevara-Pérez, & Pérez, 2020; Freytez & Márquez, 2021). The result of the comparison indicates that there is influence of COD concentration and cycle duration on the COD removal efficiency. Another aspect that could influence the COD removal efficiency could be associated to the settling properties of the granular biomass due to the fact that its components include some presence of different concentrations of Mg^{2+}, Ca^{2+}, and Ba^{2+} salts. The presence of any of these cations has increased the flocculation ability of the biomass and improved the biomass settleability in biological reactors such as the UASB as a result of the content mainly of calcium in the waste (Lettinga, Van-Velsen, Hobma, De-Zeeuw, & Klapwijk, 1980) as a part of the hydrid anaerobic reactors (suspended growth system-attached growth system) (Márquez, Maldonado, Guevara, Rey, & Pérez, 2021).

Table 3. Experimental conditions of SBR operation.

N°	Authors	IS			BP	IC mg l^{-1}			RV (l)	BT	RE	CD (h)								
		D	I	S		1	2	3				1	2	3	4	5	6	7	8	9
1	Irvine *et al*., 1979	1			2	238	215	25.8	5	1	90	6,10				12,16		1	1	
2	Alleman and Irvine, 1980			1	2	200	2500	0	7.2	1	80	2				4		1	1.5	8.5
3	Alleman and Irvine, 1980			1	1	200	2500	0	7.2	1	90	2				6	8	0.5	0.5	
4	Silverstein and Schroeder 1985			2	1	500000	3994000	50000	4	1	80	2	3		3	1	0.33	1	0.17	9.5
5	Manning and Irvine, 1985			3	2	330000	1500000	27000	3.6	1	70	2				4		1	1	8
6	Beun *et al*., 1999			4	2	83000	119000	4000	2.5	1	80	0.03				3		0.03	0.02	3
7	Carucci *et al*., 1999		1		1	1300		200	5	1	80					3.5		1.5	1	6
8	De-Silva and Rittmann 2000	1				500	75	0	4	1	80									8
9	Beun, Heijnen and Van Loosdrecht, 2001			5	2	25000	11900	40	3	2	70-80									3
10	Di-Iaconi *et al*., 2002		1		2	3500	1500	250	16	1	97-98	0.06				1	6.81	0.02	0.1	8
11	Arrojo, Mosquera-Corral, Garrido and Méndez, 2004			4	1	3000	1000	200	2.5	2	70-90	0.05				2.85		0.01	0.08	3
12	Farabegoli,Carucci, Majone and Rolle, 2004		1		1	845	3200	97	5	1	70					3.5		1.5	1	6
13	Tünay, Kabdasli and Guen, 2004		1		2	2600	4370		8	1	85	0.5, 2, 3				6, 8, 18		11, 5, 2	0.5, 1	8, 12, 24

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N°	Authors	IS			BP	IC mg l-1			RV (l)	BT	RE	CD (h)								
		D	I	S		1	2	3				1	2	3	4	5	6	7	8	9
14	Lefebvre *et al.*, 2004		1		2	2200	12000	150	10	1	92-96	0.5				22		1	0.5	24
15	Lefebvre *et al.*, 2004		1		3	2500	1500		5	1	77									
16	De-Kreuk *et al.*, 2005			6	1	16000	8500	1800	3	2	95	1				1.86		0.05	0.08	3
17	Palma-Costa y Manga, 2005	2			1	158	350	63.1		1	80.00									8
18	Su and Yu, 2005			2	2	1250	3000		2.2	2	90	0.1				3.66		0.08	0.08	4
19	Su and Yu, 2005	2			2	2000	5		3.8	2	80	0.1				3.66		0.08	0.08	4
20	Ganesh *et al.*, 2006		1		2	1910	1900	120	8	1	80-85	6,1				7,22		0.75, 0.75	0.25, 0.25	12,24
21	Su and Yu, 2006			2	2	3000	1200	200	3.8	2	80-90	0.08				5.75		0.08	0.08	6
22	De-Kreuk *et al.*, 2006			6	1	16000	1800	1200	5	2	90	1				1.86		0.13		3
23	Murat *et al.*, 2006		1		1	2700		15.8	3	1	60-75	1	1.5			8.5		2	2	12
24	Ni and Yu, 2008			7	2	800	1500			2		0.05				4		0.08	0.08	4.20
25	Insel *et al.*, 2009		1		2	2200	820	125	2	1	60-85									8
26	Loaiza-Navía and Fall, 2010	2			2	5804000	260000	267000	27.5E6	1	70.00									
27	Pire-Sierra *et al.*, 2010		1		1	25000		2800	500	1	85	1.25	3,1.8			7,4.2		0.5		12,8
28	Pire-Sierra *et al.*, 2010		1		1	1713, 3		111, 4	4	1	40-85	1.25	3, 1.8			7, 4.2		0.5		12,8
29	Vázquez-Padín *et al.*, 2010			8	2	55000		10000	4	2	30.00	0.05				2.85			0.01	0.08
30	El-Sheikh, Saleh, Flora, and AbdEl-Ghany, 2011		1		3	13500	6700	1016	1.5	1	80									
31	Jungles *et al.*, 2011			8	2	2800	190		94	1	92	0.01				2.8		1	0.03	3
32	Pire *et al.*, 2011		1		2	2510	960	75.3	125	1	57.40									24
33	Isanta *et al.*, 2012		2		2	400	5	40	4	2	70-85	0.05				2.85			0.01	3
34	Del-Rio *et al.*, 2012	2			2	1750	700	185	100	2	60-95	0.05				2.85			0.01	3
35	Kocijan and Hvala, 2013	2			1	3800		8.18	2.5	1		0.6				4		2.33	1	8
36	Isanta *et al.*, 2013	2			2	600	5	103	71	2	70-85	0.01				2.8		1	0.03	3
37	Carrasquero *et al.*, 2014		1		1	1901		140	1.5	1	50-90									10

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N°	Authors	IS			BP	IC mg l^{-1}			RV (l)	BT	RE	CD (h)								
		D	I	S		1	2	3				1	2	3	4	5	6	7	8	9
38	Carrasquero *et al.*, 2014		3		1	4790		273.8	2	1	89.00	0.25				6,7		1.5, 1.75	1.5, 2.25	10, 12
39	Ni *et al.*, 2014	2			1			1250000	2	1	80									6,10
40	Luo *et al.*, 2014			8	1	300		30	1.4E6	1	84-98	2.5	2.5			5		0.5		8
41	Freytez *et al.*, 2015		1		2	5584		80.33	10	2	59-33	0.25				23.61		0.03	0.1	24
42	Pire-Sierra, Cegarra-Badell, Carrasquero-Ferrer, Angulo-Cubillan, and Díaz-Montiel, 2016		1		1	1546		121	3	1	80-99,6	0.08				11		0.75	0.25	12
43	Freytez *et al.*, 2019a; Freytez *et al.*, 2019b; Freytez *et al.*, 2019c; Freytez and Márquez, 2020; Freytez *et al.*, 2020		1		1	5584		80.33	3	2	50-60	0.05				5.83, 23.83		0.03		6,24

IS: Input substrate; BP: Biological process; IC: Input concentration; RV: Reactor volume; BT: Biomass type; RE: Reactor efficiency; CD: Cycle duration; T: temperature.

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Table 4. Code of variables of SBR operation.

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Input Substrate (IS)									
Domestic (D)	Rural	Municipal							
Industrial (I)	Tannery	Porcine	Birds						
Synthetic (S)	Peptone and carbohydrate mixture	Sucrose, Bactopeptone and inorganic nutrients	CH_3COONa, Glucose, Amino acids	Ethanol, NH_4Cl, K_2HPO_4, $MgSO_4$, $CaCl.2H_2O$, EDTA	CH_3COONa, $MgSO_4.7H_2O$, K_2HPO_4	CH_3COONa, $MgSO_4.7H_2O$, KCl, NH_4Cl	CH_3COONa, Propionate	CH_3COONa, $3H_2O$, NH_4Cl, $NaHCO_3$	
Biological process (BP)	Mixed	Aerobic	Anaerobic						
Influent concentration (IC)	COD mg l^{-1}	VSS mg l^{-1}	NH_4^+ mg l^{-1}						
Biomass type (BT)	Suspended	Granular							
Cycle duration (CD)	Filling time	Anoxic	Anaerobic	Anoxic	Oxic	Anoxic	Settling	Effluent withdrawal	Total

When comparing the treatments for the individual oxic sequence and the mixed one (anaerobic-oxic), a slight difference was found in the COD removal efficiency and SUR (T1 and T3, T2 and T4). This result leads to either treatment T1 to T4 would be a treatment option, although the T3 would be the best choice because of the lower energy consumption. The SUR negative sign is signifying the decrease of the substrate with time due to the action of the biodegradation exerted by the granular biomass (Table 1).

By comparing SUR in T1 to T4 with another biological reactor (Table 1), with respect to the upflow anaerobic filters (UAF) separated in two phases (UAF-2SS) and three phases (UAF-3SS), several models were calibrated and validated to determine the kinetic parameter of substrate maximum utilization rate (k_m). Among the proposed models, Márquez *et al*. (2021) calibrated a modification of the Monod's equation finding ($r_{(m,S)}$) that took values for UAF-2SS corresponding to -146.51 mg l^{-1} h^{-1} and fro UAF-3SS of -195.38 mg l^{-1} h^{-1}. In the same study, Márquez *et al*. (2021) calibrated with another dataset a first coupled model superimposing equations proposed by Van't Hoff (1884); Shulze (1960); Germain (1966), and Albertson and Davis (1984) obtaining k_m for UAF-2SS of -0.27 to -0.56 mg COD mg VSS^{-1} day^{-1} (-15.63 mg l^{-1} h^{-1}) as well as UAF-3SS of -2.05 to -4.97 mg COD mg VSS^{-1} day^{-1} (-55.91 mg l^{-1} h^{-1}), respectively. In addition, Márquez *et al*. (2021) developed a second coupled model calibration supported in the combination of the Monod's equation (Monod, 1942), Velz's Law (Velz, 1948) and Fick's Law (Velz, 1948) distinguishing between vertical and horizontal molecular diffusion and resulting as similar response in terms of k_m for UAF-2SS of -0.90 to -0.93 mg COD mg VSS^{-1} day^{-1} (-25.65 mg l^{-1} h^{-1}) as well as UAF-3SS of -8.19 to -8.84 mg COD mg VSS^{-1} day^{-1} (-246.78 mg l^{-1} h^{-1}), respectively. The $r_{(m,s)}$ values reported by UAF-3SS were within the interval of those estimated for T2 and T4 (Table 1) confirming a similar performance of the biofilm with respect to the biomass in SBR for a wastewater containing recalcitrant and inhibitory substances (Maldonado-Maldonado, Márquez-Romance, Guevara-Pérez, Pérez, & Rey-Lago, 2018a; Maldonado-Maldonado, Márquez-Romance, Guevara-Pérez,

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Pérez, & Rey-Lago, 2018b; Maldonado-Maldonado, Márquez-Romance, Guevara-Pérez, Pérez, & Rey-Lago, 2020; Maldonado-Maldonado, Márquez-Romance, Guevara-Pérez, Pérez, & Rey-Lago, 2021; Márquez, Maldonado, Guevara, Rey, & Pérez, 2021a; Márquez, Maldonado, Guevara, Rey, & Pérez, 2021b).

In another study, Maldonado *et al*. (Maldonado *et al*., 2018a; Maldonado *et al*., 2018b) calibrated and validated models based on a coupled model derived from the equations proposed by Germain (1966), and Albertson and Davis (1984) for UAF-2SS and UAF-3SS operating under the reactors containing a mean volatile suspended solids (VSS) concentration of 670 mg VSS l^{-1}, finding mean values for k between -0.88 and -1.13 mg COD mg VSS^{-1} day^{-1} (-31.54 mg l^{-1} h^{-1}) likewise -$6x10^{-4}$ and -1.32 mg COD mg VSS^{-1} day^{-1} (-36.85 mg l^{-1} h^{-1}), respectively. A second coupled model by Maldonado *et al*. (2020) involved the combination of equations proposed by Vant Hoff (1884), Schulze (1960), Germain (1966), and Albertson and Davis (1984) finding mean values for k associated to UAF-2SS of -0.20 mg COD mg VSS^{-1} day^{-1} (-5.6 mg l^{-1} h^{-1}) as well as UAF-3SS of -4.49 mg COD mg VSS^{-1} day^{-1} (-125.34 mg l^{-1} h^{-1}), respectively. A third coupled model by Maldonado *et al*. (2020) included the combination of equations obtained by Schulze (1960), Germain (1966), and Albertson and Davis (1984) finding mean values for k associated to UAF-2SS of -0.34 mg COD mg VSS^{-1} day^{-1} (-9.49 mg l^{-1} h^{-1}) as well as UAF-3SS of -1.01 mg COD mg VSS^{-1} day^{-1} (-28.19 mg l^{-1} h^{-1}), respectively. A fourth coupled model by Maldonado *et al*. (Maldonado *et al*., 2020; Maldonado *et al*., 2021) included the combination of equations obtained by Phelps (1944) and

Stack (1957) that explain intra and extracellular transport and transformation processes, finding mean values for k associated to UAF-2SS of -16 and -19.33 mg COD mg VSS^{-1} day^{-1} (-539.62 mg l^{-1} h^{-1}) as well as UAF-3SS of -1.56 and -2.2 mg COD mg VSS^{-1} day^{-1} (-61.41 mg l^{-1} h^{-1}), respectively. These values for $r_{(m,S)}$ indicate that the COD biodegradation for UAF-2SS was lower than UAF-3SS. In comparison with the values reported in Table 1, r_{ms} for UAF.3SS was more intense and rapid than SUR for SBR under oxic phase in T1 and T4 in an order varying between 2 and 4 times.

Comparison of the FED1 results with other studies

Based on comparison of FED1 results with 16 studies on SBR treating tannery wastewater in the period 1979-2019, which were carried out by Irvine *et al*. (1979); Su and Yu (2005); Su and Yu (2006); Del-Rio *et al*. (2012); Isanta *et al*. (2012); Isanta *et al*. (2013); Di-Iaconi *et al*. (2002); Tuenay, Kabdasli, and Guen (2004); Lefebvre *et al*. (2004); Ganesh *et al*. (2006); Insel, Görgün, Artan, and Orhon (2009); Alleman and Irvine, 1980; Zheng, Yu and Sheng (2005); Jungles *et al*. (2011); Freytez *et al*. (2019a); Freytez *et al*. (2019b):

- The COD in the influent of 5 873 mg l^{-1} to the SBR in T1 and T2 (Table 1) resulted 4.7 times higher than the mean value used in other studies.

- The COD removal and utilization rate values found in T1 to T4 (Table 1), resulted in a low occurrence frequency less than 10 % of the time.

Some of causes to obtain these results in the FED1 are because of absence of organisms with the required biodegradation capacity, or their presence at very low concentrations, lack of primary substrate to promote cometabolism, lack of an essential nutrient or electron acceptor, toxic concentrations of the compound to be degraded producing enzyme inactivation (Guevara, 2016). For instance, scarce COD removal and significant nitrification have been found in the oxic stage of the SBR treating tannery wastewater by Carucci *et al*. (1999) (Table 3). These results indicate a low activity of the heterotrophic biomass and high of the autotrophic biomass, respectively. A similar behavior of autotrophic and heterotrophic biomass has been reported in other studies, where nitrification was fully achieved in a SBR being fed with tannery wastewater containing a chloride concentration of around 5 000 mg l^{-1} (Murat, Atesş-Genceli, Tasşli, Artan, & Orhon, 2002) (Table 3), and total chromium of 95 mg l^{-1} (Ganesh *et al*., 2006) (Table 3).

Treatments using suspended biomass

Comparison of removal of the organic and nitrogenated substrates in SBR using suspended biomass

In the 12 treatments obtained from experimental design No. 2, whose values and statistical parameters are shown in Figure 5 and Table 2, the following findings and hypotheses have been found:

In the first anoxic stage, there are mostly autotrophic (nitrification) and heterotrophic (anaerobic digestion processes) biomass activities but not enough organisms to generate a sufficient amount of enzymes to biodegrade the entire substrate. The COD removal efficiency varied between 47 and 51 % (Table 2) possibly due to anaerobic digestion processes such as complex hydrolysis (particulate organic materials from proteins, carbohydrates and lipids), fermentation of amino acids (proteins), sugars (carbohydrates) and fatty acids (lipids), aceticlastic or reductive methanogenesis (Pavlostathis & Giraldo-Gomez, 1991). The removal efficiency of nitrogenated substrates resulted in TKN of 28 % and of 18 % for NH_4^+-N (Figure 5). The particular case of increase in COD and TKN in the anoxic stage I (Figure 5) is possibly due to the lack of a network of microbial consortia to carry out cometabolic commensalism where one population benefits from the crumbs of another to achieve mineralization of recalcitrant compounds (Guevara, 2016). The highest removal of COD (51 %) and TKN (50-66 %), as well as nitrification-denitrification in the treatment of tannery wastewater was

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reported in the anoxic stage for SBR operating in an anoxic-oxic sequence by Carucci, Chiavola, Majone and Rolle (1999). The nitrification-denitrification processes were fully achieved in a SBR operating in an anoxic-oxic aeration sequence for a 12-hour cycle fed by tannery wastewater, containing 25-43 mg l^{-1} of Total Chromium and 4 638-6 075 mg l^{-1} of Chloride (Murat *et al*., 2002). These results indicate that the nitrification-denitrification processes are carried out by a facultative biomass consisting of chemoheterotrophic and chemoautotrophic organisms coexisting within the SBR to treat tannery wastewater under an anoxic-oxic sequence. The increase in the performance in the removal of organic substrates (COD) and nitrogen substrates from tannery wastewater would be achieved by stimulating the growth of heterotrophic biomass (biostimulation) by introducing macro and micronutrients to achieve the fermentation of the organic and inorganic substrates in the anoxic I stage of the mixed aeration sequence (anoxic I-oxic-anoxic II) (Guevara, 2016).

The reduction of NH_4^+-N was substantial within the first anoxic stage (Figure 4d), where it is observed that the NH_4^+-N utilization rate (r_s) reached values between -1 and -2 mg l^{-1} h^{-1}, 5.5 to -7.5 mg l^{-1} h^{-1} (oxic) and -1.5 to -3.5 mg l^{-1} h^{-1} (anoxic II CND and SND). The mean values of NH_4^+-N maximum utilization rate were varying between -0.30 (anoxic I-SND) and -1.12 mg l^{-1} h^{-1} by calibrating the modified Monod equation (Anoxic I CND) (Freytez *et al*., 2019c; Freytez *et al*., 2020). These SUR values resulted similar to those found in the present study, where the most significant NH_4^+-N occurred in the first anoxic stage.

The reduction of TKN was significant within T5 (Table 2), where it is observed that the TKN utilization rate (r_S) reached values between -5.3 and -9.1 mg l^{-1} h^{-1}. The mean value of TKN maximum utilization rate was varying between -2 to -4 mg l^{-1} h^{-1} (Anoxic I), -4 and -5 mg l^{-1} h^{-1} (oxic), -3 to -5 mg l^{-1} h^{-1} (Anoxic I CND) and -5 to -7 mg l^{-1} h^{-1} (anoxic I CND) (Figure 4f), being similar values for the microbial performing in the TKN removal within SBR.

In the aeration stage mostly occurs that COD increases (Figure 5), TKN (62-84 %) (Figure 5, Table 2) and NH_4^+-N (71-91 %) are removed (Figure 5, Table 2). The increase in COD could be due to the nitrification-denitrification process or due to the action of some toxic component preventing the activity of some extracellular enzymes (Guevara, 2016). The removal of the TKN suggests that there are residuals in the liquid medium of the SBR of reaction intermediates, possibly associated with the denitrification process by aerotolerant or facultative heterotrophic organisms (Metcalf & Eddy, 1995). The removal efficiency of NH_4^+-N is 96.95 % confirming the occurrence of nitrification by heterotrophic organisms. There is a low amount of heterotrophic microorganisms to biodegrade organic substrates (COD) and the required amount of autotrophic organisms to carry out the nitrification process. Some similarities have been reported in other studies, nitrification was achieved entirely in a SBR being fed with tannery wastewater containing a Chloride concentration of around 5 000 mg l^{-1} (Murat *et al*., 2002), and total Chromium of 95 mg l^{-1} (Ganesh *et al*., 2006). No removal of COD and significant nitrification were found in the oxic stage of SBR treating tannery wastewater by Carucci *et al*. (1999). These results indicate a low activity of the heterotrophic biomass and a high activity of the autotrophic biomass, respectively.

In the second anoxic stage, the variables associated with COD (4-8 %) (Figure 5), TKN (13-48 %) (Figure 5), NH_4^+-N (59-83%) (Figure 5), are increased. The increase may be due to concentrations remaining from other cycles in the SBR. The response pattern observed between treatments regarding the performance of biomass in the SBR may be due to the lack of an essential nutrient or electron acceptor, lack of primary substrate to promote cometabolism, presence of inhibitory substances and biorecalcitrants (Guevara, 2016). These causes would lead to a reduced number of heterotrophic biomass to carry out fermentative metabolism of organic substrates motivated to a low availability of inorganic nutrients in traces such as metals (Fe, Ni, Co, Mo, and Zn) (Guevara, 2016). Likewise, the accumulation of organic and nitrogenous substrates may be due to the existence of the substrate but there is no induction of enzymes in the microorganisms present in the mixed liquor (Guevara, 2016), due to very low concentrations of the organisms with the capacity of biodegradation required to give rise to a group of intracellular biochemical reactions of anaerobic digestion (hydrolysis, fermentation, anaerobic oxidation, acidogenesis and methanogenesis) (Pavlostathis, & Giraldo-Gomez, 1991).

The SUR negative sign in the FED2 for a cycle duration of 12 h is signifying the decrease of the substrate with time due to the action of the biodegradation exerted by the suspended biomass (Table 2).

SBR treatments using suspended biomass in different filling times

Most of the studies with suspended biomass and mixed biological process sequence have been used in SBR to evaluate a single filling time within the cycle duration (Carucci *et al*., 1999; Murat *et al*., 2002; Freytez *et al*., 2019a; Freytez *et al*., 2019b). The filling time varied between 0.22 h and 1.5 h, occurring in an anoxic condition. It was found that COD and NOx-N removals occurred for a removal efficiency ranging 60-80 % and 40-50 % in the first anoxic phase, respectively, during the filling time. The values of the utilization rate and removal efficiency of organic and nitrogenous substrates were similar in the current and other studies (Table 2), indicating that the variation of the SBR filling time has a low influence on the biodegradation of the substrates in the reaction phase.

Comparison of removal rate of granular and suspended biomass

As a sample, in the oxic phase of SBR, the COD removal rate in a SBR for cycle durations of 6, 12 and 24 hours using suspended and granular biomass is shown in Figure 6a. It is observed that the COD removal rate by the suspended biomass and a cycle duration of 12 hours takes similar values under fast filling times (-48 mg l^{-1} h^{-1}), slow (-73 mg l^{-1} h^{-1}) and stages (-60 mg l^{-1} h^{-1}). The COD removal rate by granular biomass under aerobic conditions and a combination of 25% anaerobic and 75% aerobic for cycle durations of 24 and 6 hours result in the former (Figure 6a): (-105 mg l^{-1} h^{-1} , -203 mg l^{-1} h^{-1}); in the second: (-119 mg l^{-1} h^{-1}, -190 mg l^{-1} h^{-1}).

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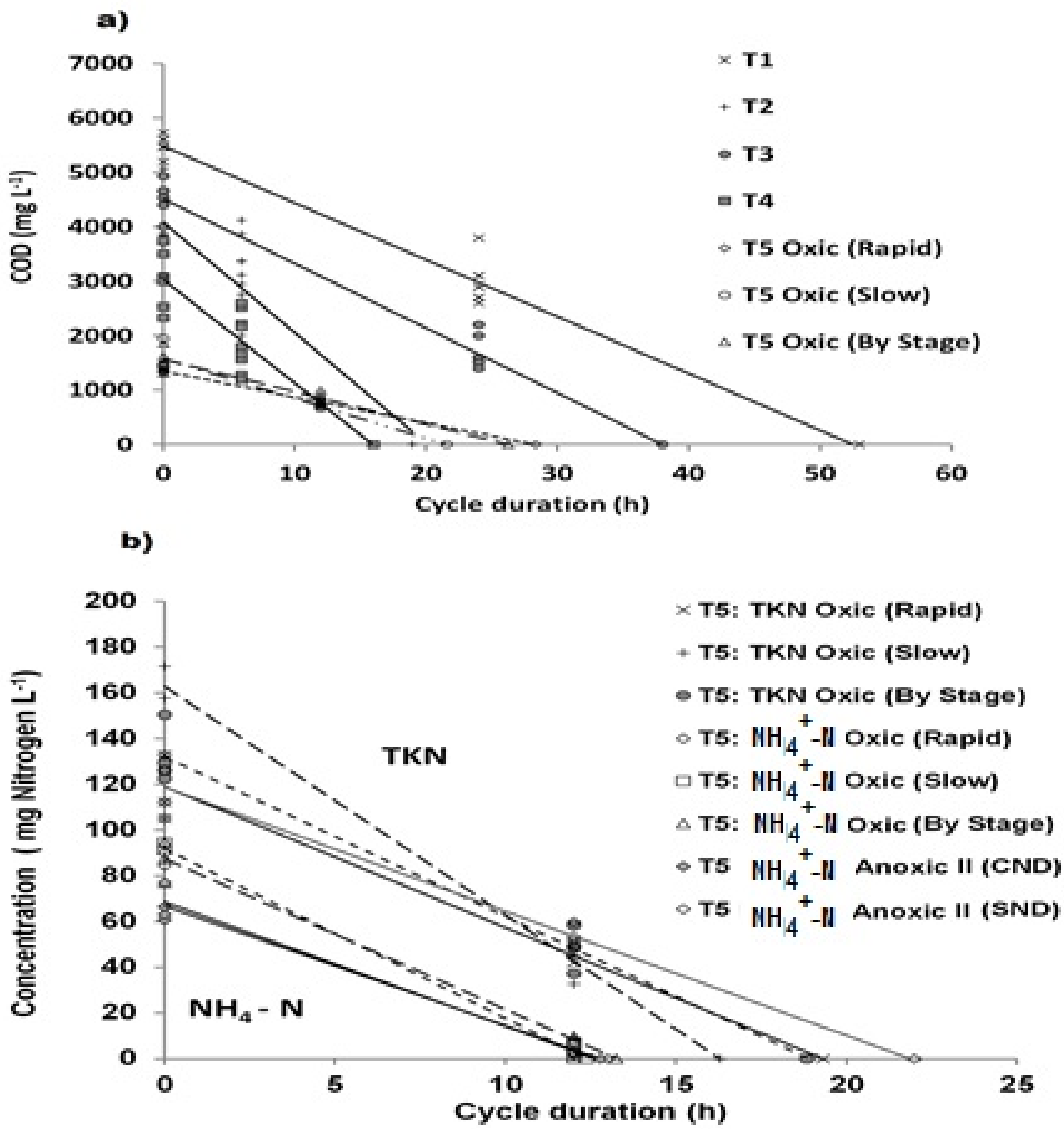

Figure 6. Rate of removal of COD and ammonium (NH_4^+-N) within the SBR cycle for cycle times of 12 hours in the stages of removal of carbonated organic matter (aeration) and conventional nitrification-denitrification (CND) applying filling times: Rapid, slow, by stages. Source: Own elaboration.

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From Figure 6a, the following aspects are inferred: 1) Granular biomass under anaerobic-oxic phases has a COD removal performance that varies between 1.5 and 2 times, which is higher than the performance of biomass suspended under aerobic conditions; 2) Granular biomass has shown a COD removal rate for a cycle time of 6 hours in the order of 2 times greater than COD removal rate for cycle duration of 24 hours. In the first, the maximum COD removal rate in the three sequential phases using suspended biomass occurs in the first anoxic stage. In the later, there is randomness in the response of the granular biomass for cycle duration in a short period of time with respect to a longer cycle of duration for SBR operation.

In Figure 6b, it can be seen that the NH_4^+-N removal rate and total Kjeldahl nitrogen (TKN) removal rate in a SBR for cycle durations of 12 hours using biomass suspended in the oxic phase and anoxic II (CND) applying slow, rapid, by stage, filling times. It is observed that the NH_4^+-N removal rate by the suspended biomass is similar under rapid, slow and stage filling time. TKN remains above the NH_4^+-N in a proportion close to 2 times higher for all treatments.

Conclusions

The tannery wastewater was treated with granular biomass in the SBR biological process under two types of sequences: Oxic and anaerobic-oxic obtaining similar removal in COD, requiring cycle durations of 24 h to achieve stabilization in the COD removal rate.

The COD removal was increased in two times for cycle durations increased from 6 h to 24 h. COD removal is influenced by inhibitory and recalcitrant matter contained in the tannery wastewater for the biological activity in SBR operation, lack of an essential nutrient or electron acceptor, toxic concentrations of the compound to be degraded producing enzyme inactivation, reaching efficiency values that varied between 30 and 40 %.

For the granular biomass performance, the trend of COD removal rate in the oxic system was a slightly change, taking a value around -100 to -295 mg l^{-1} h^{-1} compared to the anoxic-oxic system, which showed values from -46 mg l^{-1} h^{-1} to -53 mg l^{-1} h^{-1} .

The TKN and NH_4^+-N removal rates were significant in the first anoxic phase and oxic phases, respectively. This result suggests that simultaneous nitrification-denitrification is taking place in the first anoxic phase and the microorganisms are carrying out a conventional reaction of nitrification in the oxic stage.

For the removal of organic and nitrogenated substrates from the tannery wastewater, the suitable treatment in SBR would be composed by a sequential biological mixed phase (anoxic-oxic) using granular biomass.

References

Albertson, O. E., & Davis, G. (1984, October). Analysis of process factors controlling performance of plastic bio-media. *Proceedings of the 57th Annual Meeting of the WPCF*, New Orleans, LA.

Alleman, J. E., & Irvine, R. L. (1980). Nitrification in the sequenching batch biological reactor. *Journal (Water Pollution Control Federation)*, 52(11), 2747-2754. Recovered from https://www.jstor.org/stable/25040953

Álvarez, P., & Guevara, E. (2003). *Biorremediación y atenuación natural de acuíferos contaminados por sustancias químicas peligrosas*. Valencia, Venezuela: Consejo de Desarrollo Científico y Humanístico de la Universidad de Carabobo (CDCH-UC).

APHA-AWWA-WPCF, American Public Health Association, American Water Works Association, Water Pollution Control Federation. (2005). *Métodos normalizados para el análisis de las aguas potables y residuales (Standardized methods for the analysis of drinking water and wastewater)*. Recovered from https://agris.fao.org/agris-search/search.do?recordID=US9335741

Arrojo, B., Mosquera-Corral, A., Garrido, J. M., & Méndez, R. (2004). Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Research*, 38(14-15), 3389-3399. Recovered from https://doi.org/10.1016/j.watres.2004.05.002

Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A., & Heijnen, J. J. (1999). Aerobic granulation in a sequencing batch reactor. *Water Research*, 33(10), 2283-2290.

Beun, J. J., Heijnen, J. J., & Van Loosdrecht, M. C. M. (2001). N-Removal in a granular sludge sequencing batch airlift reactor. *Biotechnology and Bioengineering*, 75(1), 82-92. Recovered from https://onlinelibrary.wiley.com/doi/abs/10.1002/bit.1167

Carrasquero, S., Matos, E., Saras, F., Pire, M., Colina, G., & Díaz, A. (2014). Evaluación de la eficiencia de un reactor por carga secuencial tratando aguas residuales provenientes de un matadero de reses. (Evaluating the efficiency of a reactor by sequential loading treating wastewater from a cattle slaughterhouse). *Revista de la Facultad de Ingeniería Universidad Central de Venezuela*, 29(3), 7-16. Recovered from http://www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0798-40652014000300002

Carucci, A., Chiavola, A., Majone, M., & Rolle, E. (1999). Treatment of tannery wastewater in a sequencing batch reactor. *Water Science and Technology*, 40(1), 253-259. Recovered from https://doi.org/10.1016/S0273-1223(99)00392-3

De-Silva, D. V., & Rittmann, B. E. (2000). Nonsteady-state modeling of multispecies activated-sludge processes. *Water Environment Research*, 72(5), 554-565. Recovered from https://doi.org/10.2175/106143000X138139

De-Kreuk, M. K., Heijnen, J. J., & Van Loosdrecht, M. C. M. (2005). Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. *Biotechnology and Bioengineering*, 90(6), 761-769. Recovered from https://doi.org/10.1002/bit.20470

De-Kreuk, M. K., McSwain, B. S., Bathe, S., Tay, S. T., Schwarzenbeck, N., & Wilderer, P. A. (2006). Discussion outcomes Ede. In: Aerobic Granular Sluge (pp. 162-169). Water and Environmental Management Series. London, UK: IWA Publishing.

Del-Rio, A. V., Figueroa, M., Arrojo, B., Mosquera-Corral, A., Campos, J. L., García-Torriello, G., & Méndez, R. (2012). Aerobic granular SBR systems applied to the treatment of industrial effluents. *Journal of Environmental Management*, 95, 88-S92. Recovered from https://doi.org/10.1016/j.jenvman.2011.03.019

Di-Iaconi, C., Lopez, A., Ramadori, R., Di-Pinto, A. C., & Passino, R. (2002). Combined chemical and biological degradation of tannery wastewater by a periodic submerged filter (SBBR). *Water Research*, 36(9), 2205-2214. Recovered from https://doi.org/10.1016/S0043-1354(01)00445-6

Dold, P. L., & Ekama, G. A. (1981). A general model for the activated sludge process. In: *Water pollution research and development* (pp. 47-77). Recovered from https://doi.org/10.1016/B978-1-4832-8438-5.50010-8

El-Sheikh, M. A., Saleh, H. I., Flora, J. R., & AbdEl-Ghany, M. R. (2011). Biological tannery wastewater treatment using two stage UASB reactors. *Desalination*, 276(1-3), 253-259.

Farabegoli, G., Carucci, A., Majone, M., & Rolle, E. (2004). Biological treatment of tannery wastewater in the presence of chromium. *Journal of Environmental Management*, 71(4), 345-349.

Fernandes, H., Jungles, M. K., Hoffmann, H., Antonio, R. V., & Costa, R. H. (2013). Full-scale sequencing batch reactor (SBR) for domestic wastewater: Performance and diversity of microbial communities. *Bioresource Technology*, 132, 262-268. Recovered from https://doi.org/10.1016/j.biortech.2013.01.027

Freytez-Boggio, E., Silva-Escalona, R., Pire-Sierra, M. G., Molina-Quintero, L. R., & Pire-Sierra, M. C. (2015). Comportamiento fisicoquímico y microbiológico de un biorreactor durante la aclimatación de la biomasa granular usando efluentes de una tenería. *Agroindustria, Sociedad y Ambiente*, 3(1), 66-82. Recovered from http://revencyt.ula.ve/storage/repo/ArchivoDocumento/asa/n4/art05.pdf

Freytez-Boggio, E., & Márquez-Romance, A. M. (2021). *Modelación dinámica de los procesos de eliminación de materia orgánica y nitrógeno de efluentes de tenería usando un reactor por carga secuencial* (tesis doctoral). Universidad de Carabobo. Recovered from http://mriuc.bc.uc.edu.ve/handle/123456789/8695?show=full

Freytez, E., Márquez, A., Pire, M. C., Guevara, E., & Pérez, S. (2019a). Design, construction and evaluation of the performance of a load reactor sequential for treatment of wastewater of tannery. *Revista Ingeniería UC*. Recovered from https://www.redalyc.org/jatsRepo/707/70758484006/html/index.html

Freytez, E., Márquez, A., Pire, M. C., Guevara, E., & Pérez, S. (2019b). Operation assessment of the sequential batch reactor in tannery effluents using suspended and granular biomass. *Energía y Sostenibilidad*. DYNA, 10. Recovered from http://dx.doi.org/10.6036/ES9130

Freytez, E., Márquez, A., Pire, M. C., Guevara, E., & Pérez, S. (2019c). Nitrogenated substrate removal modeling in sequencing batch reactor oxic-anoxic phases. *Journal of Environmental Engineering. ASCE*. Recovered from https://ascelibrary.org/doi/abs/10.1061/%28ASCE%29EE.1943-7870.0001556

Freytez, E., Márquez, A., Pire, M. C., Guevara-Pérez, E., & Pérez, S. (2020). Organic and nitrogenated substrates utilization rate model validating in sequential batch reactor. *Journal of Environmental Engineering*, 146(3), 04019124. Recovered from https://ascelibrary.org/doi/abs/10.1061/%28ASCE%29EE.1943-7870.0001632.

Ganesh, R., Balaji, G., & Ramanujam, R. A. (2006). Biodegradation of tannery wastewater using sequencing batch reactor—respirometric assessment. *Bioresource Technology*, 97(15), 1815-1821. Recovered from https://doi.org/10.1016/j.biortech.2005.09.003

Germain, J. E. (1966). Economical treatment of domestic waste by plastic-medium trickling filters. *Journal (Water Pollution Control Federation)*, 192-203. Recovered from https://www.jstor.org/stable/25035484

Guevara, E. (2016). Transporte y transformación de contaminantes en el ambiente y contaminación de las aguas. Lima, Perú: Ministerio de Agricultura y Riego. Recovered from https://190.12.92.167/handle/20.500.12543/3941

Guo, J. H., Peng, Y. Z., Wang, S. Y., Zheng, Y. N., Huang, H. J., & Ge, S. J. (2009). Effective and robust partial nitrification to nitrite by real-time aeration duration control in an SBR treating domestic wastewater. *Process Biochemistry*, 44(9), 979-985. Recovered from https://doi.org/10.1016/j.procbio.2009.04.022

Insel, H. G., Görgün, E., Artan, N., & Orhon, D. (2009). Model based optimization of nitrogen removal in a full scale activated sludge plant. *Environmental Engineering Science*, 26(3), 471-480. Recovered from https://doi.org/10.1089/ees.2007.0272

Irvine, R. L., Miller, G., & Bhamrah, A. S. (1979). Sequencing batch treatment of wastewaters in rural areas. *Journal (Water Pollution Control Federation)*, 52(2), 244-254. Recovered from https://www.jstor.org/stable/25039820?seq=1#page_scan_tab_contents

Isanta, E., Suárez-Ojeda, M. E., Del-Río, Á. V., Morales, N., Pérez, J., & Carrera, J. (2012). Long term operation of a granular sequencing batch reactor at pilot scale treating a low-strength wastewater. *Chemical Engineering Journal*, 198, 163-170. Recovered from https://doi.org/10.1016/j.cej.2012.05.066

Isanta, E., Figueroa, M., Mosquera-Corral, A., Campos, L., Carrera, J., & Pérez, J. (2013). A novel control strategy for enhancing biological N-removal in a granular sequencing batch reactor: A model-based study. *Chemical Engineering Journal*, 232, 468-477. Recovered from https://doi.org/10.1016/j.cej.2013.07.118

Jungles, M. K., Figueroa, M., Morales, N., Val-del-Río, Á., Da-Costa, R. H. R., Campos, J. L., ... & Méndez, R. (2011). Start up of a pilot scale aerobic granular reactor for organic matter and nitrogen removal. *Journal of Chemical Technology & Biotechnology*, 86(5), 763-768, 2011. Recovered from https://doi.org/10.1002/jctb.2589

Kocijan, J., & Hvala, N. (2013).Sequencing batch-reactor control using Gaussian-process models. *Bioresource Technology*, 137, 340-348. Recovered from https://doi.org/10.1016/j.biortech.2013.03.138

Laurenti, R., Redwood, M., Puig, R., & Frostell, B. (2017). Measuring the environmental footprint of leather processing technologies. *Journal of Industrial Ecology*, 21(5), 1180-1187. Recovered from https://doi.org/10.1111/jiec.12504

Lefebvre, O., Habouzit, F., Bru, V., Delgenes, J. P., Godon, J. J., & Moletta, R. (2004). Treatment of hypersaline industrial wastewater by a microbial consortium in a sequencing batch reactor. *Environmental Technology*, 25(5), 543-553. Recovered from https://doi.org/10.1080/09593330.2004.9619345

Lettinga, G. A. F. M., Van-Velsen, A. F. M., Hobma, S. D., De-Zeeuw, W., & Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, 22(4), 699-734. Recovered from https://doi.org/10.1002/bit.260220402

Loaiza-Navía, J. L., & Fall, C. (2010). Modelación del proceso de lodos activados en la Planta de Tratamiento de Aguas Residuales Noreste, Apodaca, NL. *Ciencia UANL*, 13(1), 46-54.

Luo, T., Yang, M., Han, J., & Sun, P. (2014). A novel model-based adaptive control strategy for step-feed SBRs dealing with influent fluctuation. *Bioresource Technology*, 167, 476-483. Recovered from https://doi.org/10.1016/j.biortech.2014.05.117

Maldonado-Maldonado, J. I., Márquez-Romance, A. M., Guevara-Pérez, E., Pérez, S., & Rey-Lago, D. (2018a). Model development for the design of an anaerobic upflow filter separated in two and three phases. *Dyna*, 85(207), 44-53. Recovered from https://doi.org/10.15446/dyna.v85n207.69783

Maldonado-Maldonado, J. I., Márquez-Romance, A. M., Guevara-Pérez, E., Pérez, S., & Rey-Lago, D (2018b). Design, construction and modeling of upflow anaerobic filters separated in two and three phases. *Journal of Water Resources and Pollution Studies*, 3(3). Recovered from http://matjournals.in/index.php/JoWRPS/article/view/2762

Maldonado-Maldonado, J. I., Márquez-Romance, A. M., Guevara-Pérez, E., Pérez, S., & Rey-Lago, D. (2020). Models for the design of upflow anaerobic filters separated in two and three phases. *Journal of Environmental Engineering ASCE*. Recovered from https://ascelibrary.org/doi/abs/10.1061/%28ASCE%29EE.1943-7870.0001577

Maldonado-Maldonado. J. I., Márquez-Romance, A. M., Guevara-Pérez, E., Pérez, S., & Rey-Lago, D. (2021). Novel hybrid models for the design of upflow anaerobic filters separated in phases. *Journal of Environmental Quality Management Wiley*. Recovered from https://doi.org/10.1002/tqem.21769

Manning, J. F., & Irvine, R. L. (1985). The biological removal of phosphorus in a sequencing batch reactor. *Journal (Water Pollution Control Federation)*, 87-94. Recovered from https://www.jstor.org/stable/25042524

Márquez, A. M., Maldonado, J. I., Guevara, E., Rey, D. J., & Pérez, S. A. (2021). An approach to models for the design of upflow anaerobic filters. *Journal of Applied Water Engineering and Research*, 1-26. Recovered from https://doi.org/10.1080/23249676.2020.1831972

Metcalf & Eddy. (1995). *Ingeniería de aguas residuales. Tratamiento, vertido y reutilización*. Volumen II. 3ª ed. Madrid, España: McGraw Hill.

Monod, J. (1942). *Recherches sur la croissance des cultures bacteriennes*. Paris, France: Hermann & Cie.

Murat, S., Atesş-Genceli, E., Tasşli, R., Artan, N., & Orhon, D. (2002). Sequencing batch reactor treatment of tannery wastewater for carbon and nitrogen removal. *Water Science and Technology*, 46(9), 219-227. Recovered from https://iwaponline.com/wst/article-abstract/46/9/219/8118

Murat, S., Insel, G., Artan, N., & Orhon, D. (2006). Performance evaluation of SBR treatment for nitrogen removal from tannery wastewater. *Water Science and Technology*, 53(12), 275-284. Recovered from https://doi.org/10.2166/wst.2006.430

Ni, B. J., Joss, A., & Yuan, Z. (2014). Modeling nitrogen removal with partial nitritation and anammox in one floc-based sequencing batch reactor. *Water Research*, 67, 321-329. Recovered from https://doi.org/10.1016/j.watres.2014.09.028

Ni, B. J., & Yu, H. Q. (2008). Storage and growth of denitrifiers in aerobic granules: Part I. Model development. *Biotechnology and Bioengineering*, 99(2), 314-323.

Palma-Costa, M., & Manga, J. (2005). Simulación de un sistema de fangos activados en discontinuo SBR para el tratamiento de aguas residuales con altos contenidos de nitrógeno (Simulation of a system of activated sludge in discontinuous SBR for the treatment of wastewater with high nitrogen content). *Ingeniería y Desarrollo Colombia*, 18, 61-71. Recovered from http://rcientificas.uninorte.edu.co/index.php/ingenieria/article/viewArticle/2406

Pavlostathis, S. G., & Giraldo-Gomez, E. (1991). Kinetics of anaerobic treatment: A critical review. *Critical Reviews in Environmental Science and Technology*, 21(5-6), 411-490. Recovered from https://doi.org/10.2166/wst.1991.0217

Phelps, E. B. (1944). *Stream sanitation*. New York, USA: John Wiley and Sons. Inc.

Pire, M. C., Sargent, K. R., Reyes, M. F., Fuenmayor, Y., Acevedo, H., Ferrer, S. C., & Montiel, A. D. (2011). Biodegradabilidad de las diferentes fracciones de agua residual producidas en una tenería. *Ciencia e Ingeniería Neogranadina*, 21(2), 5-19. Recovered from https://www.redalyc.org/pdf/911/91123440001.pdf

Pire-Sierra, M. C., Cegarra-Badell, D. D., Carrasquero-Ferrer, S. J., Angulo-Cubillan, N. E., & Díaz-Montiel, A. R. (2016). Nitrogen and cod removal from tannery wastewater using biological and physicochemical treatments. *Revista Facultad de Ingeniería Universidad de Antioquia*, (80), 63-73. Recovered from https://www.redalyc.org/pdf/430/43047073008.pdf

Pire-Sierra, M. C., Palmero, J., Araujo, I., & Díaz, A. (2010). Tratabilidad del efluente de una tenería usando un reactor por carga secuencial. *Revista Científica*, 20(3), 284-292.

Schulze, K. L. (1960). Load and efficiency of trickling filters. *Water Pollution Control Federation- Part I*, 32(3), 245-261. Recovered from https://www.jstor.org/stable/25034098

Silverstein, J., & Schroeder, E. D. (1983). Performance of SBR activated sludge processes with nitrification/denitrification. *Journal* (Water Pollution Control Federation), 377-384. Recovered from https://www.jstor.org/stable/25041877

Spiegel, M. R., & Stephens, L. J. (2009). *Estadística*. México, DF, México: McGraw Hill Interamericana.

Sreeram, K. J., & Ramasami, T. (2003). Sustaining tanning process through conservation, recovery and better utilization of chromium. *Resources, Conservation and Recycling*, 38(3), 185-212. Recovered from https://doi.org/10.1016/S0921-3449(02)00151-9

Stack, V. T. Jr. (1957). Theoretical performance of the trickling filtration process. *Sewage and Industrial Wastes*, 29(9), 987-1001. Recovered from https://www.jstor.org/stable/25033426?seq=1

Stoop, M. L. M. (2003). Water management of production systems optimised by environmentally oriented integral chain management: case study of leather manufacturing in developing countries. *Technovation*, 23(3), 265-278. Recovered from https://doi.org/10.1016/S0166-4972(01)00117-1

Su, K. Z., & Yu, H. Q. (2005). Gas holdup and oxygen transfer in an aerobic granule-based sequencing batch reactor. *Biochemical Engineering Journal*, 25(3), 201-207.

Su, K. Z., & Yu, H. Q. (2006). A generalized model for aerobic granule-based sequencing batch reactor. 2. Parametric sensitivity and model verification. *Environmental Science & Technology*, 40(15), 4709-4713.

Tünay, O., Kabdasli, I., & Guen, O. (2004). Sequencing batch reactor treatment of leather tanning industry wastewaters. *Fresenius Environmental Bulletin*, 13(10), 945-950.

Van´t Hoff, J. H. (1884). *Etudes de dynamique chimique.* Amsterdam, The Netherlands: Frederik Muller & Company.

Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L., Méndez, R., Carrera, J., & Pérez, J. (2010). Modelling aerobic granular SBR at variable COD/N ratios including accurate description of total solids concentration. *Biochemical Engineering Journal*, 49(2), 173-184. Recovered from https://doi.org/10.1016/j.bej.2009.12.009

Velz, C. J. (1948). A basic law for the performance of biological filters. *Sewage Works Journal*, 20(4), 607-617.

Zheng, Y. M., Yu, H. Q., & Sheng, G. P. (2005). Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor. *Process Biochemistry*, 40(2), 645-650. Recovered from https://doi.org/10.1016/j.procbio.2004.01.056