

Article

Sequencing Batch Reactor: A Sustainable Wastewater Treatment Option for the Canned Vegetable Industry

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Abstract: The treatment of wastewater from the food processing industry, such as canned soup production, presents challenges due to its high organic load and limited nutrient concentrations. This study evaluated the efficiency of a sequencing batch reactor (SBR) in the removal of organic matter, color, turbidity, and nutrients under different solid retention times (SRTs) and operational cycle times (OCTs). The reactor operated with SRTs of 15 and 25 days and an 8-h cycle, and parameters such as COD, BOD₅, color, turbidity, nitrogen content, and phosphorus content, as well as COD fractionation were analyzed to assess biodegradability. The results showed high removal rates of organic matter, with 84.8% COD and >90% BOD₅, revealing that 54.3% of the COD was readily biodegradable. Significant reductions in color (72.3%) and turbidity (83.3%) were achieved, improving the quality of the treated effluent. Nitrogen removal occurred primarily through assimilation due to the absence of anoxic conditions, while phosphorus was also removed via biomass assimilation. The addition of macronutrients did not significantly influence treatment efficiency, reducing the need for additional inputs and operational costs. This study demonstrates the flexibility and effectiveness of the SBR in treating wastewater with a high organic load and low nutrient concentrations, highlighting its ability to produce a high-quality effluent suitable for discharge or reuse. The novelty of this work lies in combining COD fractionation analysis, nutrient removal mechanisms, and water quality parameters, providing key insights for optimizing biological processes in industrial contexts.

Keywords: sustainable wastewater treatment; sequencing batch reactor (SBR); chemical oxygen demand; vegetable processing plant effluents



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1. Introduction

The scarcity of freshwater resources for human activities is a significant concern. Currently, at least 20% of global water consumption is attributed to industrial use, a figure that is expected to quadruple by 2050. Studies indicate that the per capita water consumption varies across countries and regions, and is influenced by factors such as culture, climate, and lifestyle [1]. The disposal of waste generated by industrial activities and pollutants is a key focus in the pursuit of sustainable economic growth, with the food processing sector playing an important role in achieving the Sustainable Development Goals (SDGs) outlined in the 2030 Agenda. Issues such as increasing water stress and wastewater generation from industrial sectors necessitate effective water management strategies [2].

Food processing industries produce a wide range of products to meet the dietary needs of a growing population. Research indicates that approximately one-third of national freshwater resources are used in food production and processing. Wastewater discharge volumes also vary across industries producing different types of food products. Food processing sectors, including dairy, beverage, grocery, and fruit and vegetable processing, produce valuable edible products and by-products but also generate harmful waste [3]. For instance, most wastewater from beverage industries is generated during the washing and rinsing of cans, cleaning equipment, and maintaining cleanliness within facilities [4].

If released untreated, food processing wastewater can lead to oxygen depletion and eutrophication in water bodies. Some types of wastewaters are also colored and cloudy, which blocks light and hinders photosynthesis [5,6].

Wastewater from the food processing industry exhibits unique characteristics that distinguish it from other types of wastewaters in terms of both quality and quantity. In terms of quality, it often has high concentrations of soluble organic compounds that must be treated to reduce pollution before discharge, especially given the increasing regulations on effluent control and disposal [7,8]. Common characteristics of effluents from food industries include the chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved solids, phosphates, nitrates, surfactants, fats, oils, and intense color with a fluctuating pH [9–13]. Regarding quantity, the generation of wastewater in this industry is highly dependent on production volumes and processing methods, often resulting in intermittent discharges with high flows during specific times of the day [14,15]. These features make food processing wastewater require specialized treatment methods that may not be applicable or necessary for other types of industrial or domestic wastewater. In some cases, wastewater discharges account for up to 70% of the total water used in the industry, ranging from 0.2 to 10 m³/t of product [16,17].

Wastewater treatment methods generally include physical, chemical, biological, or combined processes. Biological wastewater treatment is a sustainable option because it requires fewer chemicals and produces fewer toxic by-products. On an industrial scale, it is economically feasible and widely applicable. Activated sludge (AS) is a common biological treatment method primarily used for domestic wastewater that can remove organic contaminants and nutrients by maintaining anaerobic–anoxic–aerobic conditions [18,19]. A sequencing batch reactor (SBR) is a variation of activated sludge treatment and is widely used for biological wastewater treatment. SBRs can maintain continuous low loading rates through simple cycle adjustments, which optimize the ratio of substrate to microorganisms and prevent sludge bulking through endogenous respiration, a feature that is not available in conventional activated sludge systems [19–21]. SBRs have proven effective for treating industrial effluents in full-scale and laboratory-scale applications, including landfill leachate [22]; municipal [23], slaughterhouse, and meat processing wastewater [24,25]; textile wastewater [26,27]; tannery wastewater [28]; seafood processing and organic food wastewater [29]; and for bioenergy and biopolymer production by an enriched biomass [30–32].

The objective of this study was to evaluate the efficiency of an SBR operated under different cell retention times and operational cycle times for treating wastewater from a canned soup processing plant. Additionally, this study aimed to determine the organic matter fractions in the effluent and analyze the degradation kinetics within the reactor.

The novelty lies in its comprehensive evaluation of a sequencing batch reactor (SBR) applied to wastewater treatment in the food processing industry, specifically under conditions of a high organic load and limited nutrient availability. Unlike previous works, this research integrates the analysis of COD fractionation, identifying the readily biodegradable fraction, with an in-depth assessment of nutrient removal mechanisms, including nitrogen and phosphorus assimilation. Additionally, it highlights the reactor's ability to achieve

significant reductions in color and turbidity, improving effluent quality without the need for external macronutrient supplementation. This approach not only optimizes the biological treatment process but also provides practical insights for reducing operational costs and enhancing the sustainability of wastewater management in industrial settings.

2. Materials and Methods

2.1. Collection of Effluents

Effluents were collected from a vegetable processing plant dedicated to canned soup production located in the plains of Maracaibo, Jesús Enrique Lossada municipality, Zulia state, Venezuela. The collection process followed the protocols established in the Standard Methods [33], using Method 1060 for sample collection and preservation. Samples were manually collected via simple random sampling at manholes located at the effluent discharge outlet. Monthly samples were obtained in clean, dark-colored plastic containers with a 20–25 L capacity. These samples were transported to the laboratory, where they were characterized and stored under refrigeration at 4 °C to preserve their initial characteristics [34].

2.2. Characterization of Effluents

The effluent samples were characterized by analyzing physical and chemical parameters using the Standard Methods [33], as detailed in Table 1. The organic matter fraction (COD), nitrogen fraction (TKN), and certain kinetic constants were determined using load reactors to ensure a more comprehensive characterization of the effluents.

Table 1. Parameters measured during the characterization of effluents.

Parameter	Method No. (APHA et al.) [33]	Method Type
BOD	5210	Potentiometric
TCOD	5220	Volumetric method (closed reflux) for chloride concentrations ≤ 2000 mg/L
SCOD ¹	5220	Volumetric method (closed reflux) for chloride concentrations ≤ 2000 mg/L
PCOD ²	---	---
TKN	4500-Norg-B	Volumetric
N-NH ₄ ⁺	4500-NH ₃ -D	Volumetric
Organic N ³	---	---
NO ₂ [−]	4500-NO ₂ [−] -B	Colorimetric
NO ₃ [−]	4500-NO ₃ [−] -B	Colorimetric
TN ⁴	---	---
TP	4500-P-C	Colorimetric
P-PO ₄ ^{3−}	4500-P-C	Colorimetric
Cl [−]	4500 Cl [−] -B	Argentometric
pH	4500 H ⁺ -B	Potentiometric
Total alkalinity	2320-B	Volumetric
Total acidity	2310-B	Volumetric
Color	2120-C	Colorimetric
Turbidity	2130-B	Nephelometric
TSSs (total suspended solids)	2540-D	Gravimetric
VSSs (volatile suspended solids)	2540-E	Gravimetric
SSs (settleable solids)	2540-F	Volumetric

BOD—biological oxygen demand, TCOD—total chemical oxygen demand, SCOD—soluble chemical oxygen demand, PCOD—particulate chemical oxygen demand, TKN—total Kjeldahl nitrogen, N-NH₄⁺—ammoniacal nitrogen, N—nitrogen, NO₂[−]—nitrite, NO₃[−]—nitrate, TN—total nitrogen, TP—total phosphorous, P-PO₄^{3−}—orthophosphate, Cl[−]—chloride. Notes: 1. The soluble COD was calculated by filtering the sample through a cellulose ester membrane with a pore size of 0.45 μ m. 2. The particulate COD was estimated from the difference between the TCOD and the SCOD. 3. The organic nitrogen content was determined by subtracting the TKN content from the N-NH₄⁺ content. 4. The TN content was calculated by adding the TKN, NO₂[−], and NO₃[−] contents.

2.3. Load Reactor Description

To determine kinetic constants and COD and TKN fractions, load reactors were used with a diameter of 14.5 cm and a height of 26 cm, providing a total volume of 4 L and an

operating volume of 2 L (Figure 1). The reactor system is driven by a single-phase motor (General Electric, model WR60X165, 15 HP, 1300 rpm, New York, NY, USA) connected to a stainless-steel shaft with a four-blade propeller. In all cases, the reactor volume consisted of 30% microbial biomass and 70% wastewater, with an operating speed set to 300 rpm [26]. Compressed air was introduced through a fine-bubble diffuser (SeaStar, model HX-308-20, Beijing, China), enhancing air transfer into the wastewater to aid in pollutant breakdown.

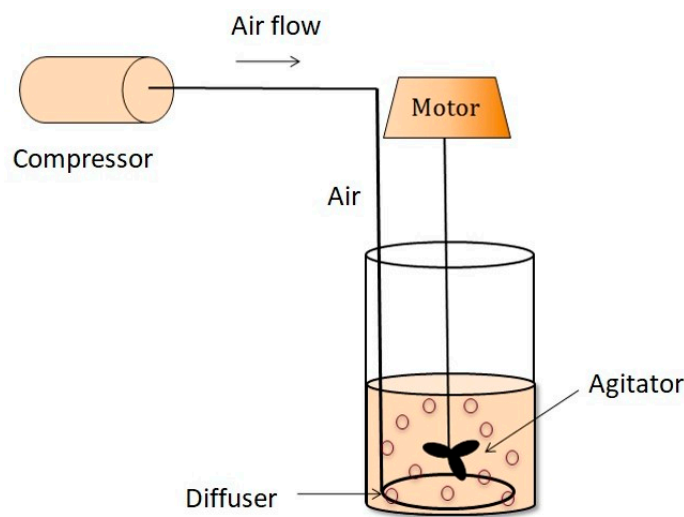


Figure 1. Batch reactor scheme.

2.4. Microbial Biomass: Origin and Treatment

The microbial biomass was obtained from an activated sludge biological reactor in a domestic wastewater treatment system. The reactor was inoculated at a 30:70 ratio by adding the collected biomass (0.6 L) to the wastewater (1.4 L), maintaining a pH range of 6.5–8.5. The mixed liquor was continuously aerated for 24 h, with a sludge retention time (SRT) of 15 days and a sedimentation time of 0.5 h before discharging the clarified effluent. Reactor stability and biomass adaptation were achieved when the COD removal efficiency exceeded 50%, and the sludge's sedimentation characteristics became stable [35].

The biomass-to-wastewater ratio, cell residence time, sedimentation time, and retention times were kept constant until the adaptation process was complete. During this process, micronutrients were added to all reactors to support the development of the microbial community, following the methodology of Di Iaconi et al. [36].

2.5. Description of Sequencing Batch Reactors

Two lab-scale cylindrical sequencing batch reactors (SBRs) with a volume of 4 L, a diameter of 14.5 cm, and a height of 26 cm were used in parallel for wastewater treatment. The working volume of each reactor was 2 L, comprising 70% wastewater and 30% adapted biomass. The reactors were equipped with fully automated fill and discharge systems using single-direction peristaltic pumps (Cole-Parmer, models 77202-60 and 77201-60, Chicago, IL, USA) controlled by timing devices (Excelline, model GTC-E-120AS9, Caracas, Venezuela) and control systems for mechanical agitation and an oxygen supply (Figure 2). Treated effluent was collected after each discharge phase.

Flexible pipes with an inlet/discharge diameter of 6 mm (Masterflex 06406-147, Chicago, IL, USA) were connected to single-flow peristaltic pumps, providing a filling/discharge flow rate of 93 mL/min. This same setup was used for extracting the mixed liquor. The mechanical stirring system consisted of a 1300 rpm single-phase 15 W AC geared motor (General Electric, model WR60X165, New York, NY, USA) connected to a 4-blade propeller within the reactor via a stainless-steel shaft. A potentiometer [37] con-

trolled the motor speed, maintaining 300 rpm. A diffuser, measuring 45 cm in length and 4 mm in diameter, was placed at the bottom of the reactor to supply air in an upward flow, ensuring a minimum dissolved oxygen concentration of 2 mg/L during the aerobic phase.

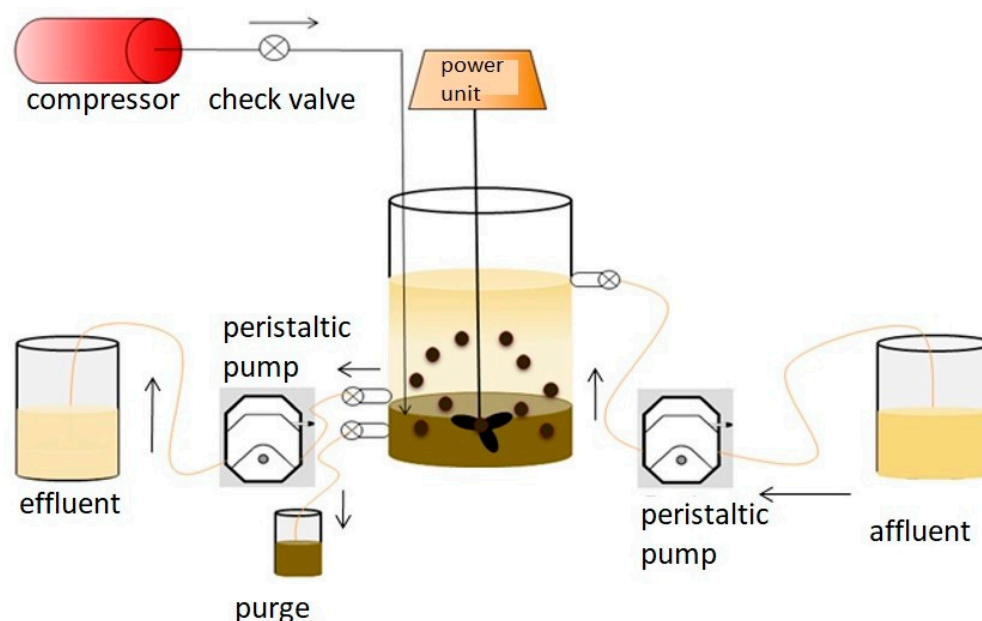


Figure 2. Description of the sequencing batch reactor (SBR).

2.6. Kinetic Constants and Organic Matter Fractionation of Effluents

To determine the biodegradable and non-biodegradable components of organic matter, the method proposed by Park et al. [38] was followed. This procedure involved measuring the total and soluble CODs of the raw wastewater and mixed liquor at the beginning and end of treatment in the batch reactor. Mathematical calculations were then applied to determine the fractions of organic matter: total biodegradable COD (TBCOD), total non-biodegradable COD (TNBCOD), easily biodegradable COD (EBCOD), slowly biodegradable COD (SBCOD), soluble non-biodegradable COD (SNBCOD), and particulate non-biodegradable COD (PNBCOD). Additionally, the heterotrophic cell yield coefficient (Y_h), the endogenous decay constant or cell death constant (K_d), and the substrate utilization rate (K) were calculated using the methods of Park et al. [38].

2.7. Operational Strategies for Nutrient Removal During the Treatment of Effluents in the SBR

A neutralization pretreatment was applied before the effluents entered the sequencing batch reactor (SBR), adjusting the pH to a range of 6.5 to 8.5 using 6.0 N NaOH. This pH range supports the growth and survival of microorganisms involved in organic matter degradation and stabilization.

2.8. Effect of Macronutrient Addition on Treatability

The effect of adding macronutrients such as nitrogen (N) and phosphorus (P) was assessed on the treatment of effluents from vegetable processing plants using the SBR. The required macronutrient levels were calculated based on the C/N/P ratio proposed by Puig et al. [39] and were set at 100:1.42:1.6. Using this ratio and the COD value from the effluent characterization, nitrogen was supplemented by adding 0.038 g/L of urea (Merck) and phosphorus was supplemented by adding 0.064 g/L of monopotassium phosphate (Merck). Two SBRs were operated in parallel under identical aerobic conditions (Table 2), with one reactor receiving vegetable processing effluent without macronutrient supplementation and the other with supplementation, as suggested by Carrasquero et al. [40].

Table 2. Operating conditions of the SBRs with and without macronutrient addition.

Parameter	SBR
Operational Cycle Time (OCT) (h)	8
Filling Time (h)	0.25
Operational Sequence (OS)	Ae ¹
Filling Type	Static
Sedimentation (h)	0.5
Discharge (h)	0.25
Sludge Retention Time (SRT) (d)	15
Hydraulic Retention Time (HRT) (h)	11.4

¹ The aerobic reaction phase lasted 7.0 h.

This experiment was conducted using a completely randomized design with two treatments—one with macronutrient addition and one without—each with 12 replicates. Control parameters, including pH and total alkalinity, were measured to monitor the biological process. For each treatment, the efficiency of organic matter removal was assessed using Equation (1), where $[COD]_o$ represents the initial concentration of total COD (mg/L) and $[COD]_f$ represents the final concentration of total COD (mg/L), as suggested by Carrasquero et al. [24]:

$$E_{COD} = \left(\frac{[COD]_o - [COD]_f}{[COD]_o} \right) * 100 \quad (1)$$

Similarly, the removal efficiencies for BOD, soluble COD, total Kjeldahl nitrogen (TKN), and total phosphorus (TP) were calculated using Equation (1) by substituting the initial and final concentrations for each parameter. These results were then compared using analysis of variance (ANOVA), and the remaining variables are presented with descriptive statistics, indicating measures of central tendency and dispersion. Statistical analyses were performed using SPSS software, version 26.0.

2.9. Performance of the SBR at Different Operational Cycle Times (OCTs) and Sludge Retention Times (SRTs)

To determine the optimal operational cycle time (OCT) and sludge retention time (SRT), two sequencing batch reactors (SBRs) were operated in parallel and independently. Each reactor maintained a fixed SRT while varying only the cycle duration, with other operational conditions held constant, as shown in Table 3. This experiment used a completely randomized factorial design with two factors—OCT duration (Factor 1) and sludge age (Factor 2)—in a 3×2 arrangement, resulting in six total treatments, as detailed in Table 3.

Table 3. Treatments conducted in the SBR for vegetable processing effluents.

Parameter	T1	T2	T3	T4	T5	T6
SRT (d)	15	15	15	25	25	25
OCT (h)	6	8	10	6	8	10
HRT (h)	8.6	11.4	14.3	8.6	11.4	14.3

Note: SRT—sludge retention time. OCT—operational cycle time. HRT—hydraulic retention time. The aerobic reaction phase lasted 5, 7, and 9 h for OCTs of 6, 8, and 10 h, respectively.

Samples were taken at the beginning, during, and at the end of each treatment. The analyses were performed in duplicate following the procedures outlined in the Standard Methods for the Examination of Water and Wastewater [33]. Additionally, the volumetric organic load (VOL) was determined using Equation (2), as proposed by Mekonnen and Leta [41]:

$$VOL = \frac{COD_{AR}}{V_U * OCT} \quad (2)$$

where COD_{AR} is the COD of the raw wastewater (mg/L), V_F is the volume of wastewater fed into each cycle (L), V_U is the useful volume of the reactor (L), and OCT is the cycle duration in the SBR (days).

The average rate of organic matter removal and the specific average rate of organic matter removal were also calculated using Equations (3) and (4) from Louvet et al. [42]:

$$\text{Organic matter removal rate} = \frac{COD_o - COD_f}{OCT} \quad (3)$$

$$\text{Specific organic matter removal rate} = \frac{COD_o - COD_f}{COD * MLVSS} \quad (4)$$

where COD_o represents the COD of raw wastewater (mg/L), COD_f is the COD of treated wastewater (mg/L), and MLVSS is the average concentration of volatile suspended solids in the mixed liquor (mg/L), while OCT denotes the cycle duration in the SBR (h).

The results of the removal of BOD, total COD, total nitrogen (NT), and total phosphorus (PT), as well as changes in color and turbidity, were compared using analysis of variance (ANOVA) and means separation through Tukey's test, which were processed with the SPSS 26.0 statistical software. Additionally, Pearson's correlation analysis was performed between the volumetric organic load (VOL) and the removed COD, as well as between the applied VOL and the organic matter removal rate.

3. Results and Discussion

3.1. Characterization of Effluents

The characteristics of the industrial wastewater were like those documented in previous studies [43–45]. The industrial effluent had average organic matter concentrations, measured as BOD and COD, of 819 and 1197 mg/L (Table 4), respectively, with a biodegradability ratio of 0.68 (BOD_{50}/COD), classifying it as biodegradable wastewater. The effluent had a soluble COD ranging from 572 to 818 mg/L, with an average of 695 mg/L, representing 58.1% of the total COD. In contrast, the particulate organic matter content ranged from 287 to 717 mg/L, with an average of 502 mg/L, representing 41.9% of the total COD.

Table 4. Physicochemical characteristics of effluents from vegetable processing.

Parameter	Unit of Expression	Value (Mean $\pm$ SD)	Maximum Established Limits ¹ [46]
BOD	mg/L	819 $\pm$ 325	60
TCOD	mg/L	1197 $\pm$ 326	350
SCOD	mg/L	695 $\pm$ 123	-
PCOD	mg/L	502 $\pm$ 215	-
Total Kjeldahl Nitrogen (TKN)	mg/L	13.1 $\pm$ 3.9	-
N-NH ₄ ⁺	mg/L	8.4 $\pm$ 2.1	-
N-NO ₂ ⁻ + N-NO ₃ ⁻	mg/L	ND	10
Total Nitrogen (TN)	mg/L	13.1 $\pm$ 3.9	40
Total Phosphorus (TP)	mg/L	6.3 $\pm$ 1.9	10
P-PO ₄ ³⁻	mg/L	4.8 $\pm$ 0.2	-
Chloride (Cl ⁻)	mg/L	138 $\pm$ 0.72	1000
pH	-	5.48 $\pm$ 0.54	6–9
Total Alkalinity	mg CaCO ₃ /L	44 $\pm$ 7	-
Total Acidity	mg/L	854 $\pm$ 200	-

Table 4. Cont.

Parameter	Unit of Expression	Value (Mean $\pm$ SD)	Maximum Established Limits ¹ [46]
Color	UC Pt-Co	113 $\pm$ 71	500
Turbidity	NTU	83 $\pm$ 23	-
Sedimentable Solids (SSs)	mL/L	2.87 $\pm$ 1.33	1
Total Suspended Solids (TSSs)	mg/L	828 $\pm$ 153	80
Volatile Suspended Solids (VSSs)	mg/L	245 $\pm$ 10	-
Total Solids (TSs)	mg/L	3182 $\pm$ 857	-
BOD/TCOD	-	0.68	-
TCOD/N-NH ₄ ⁺ /P-PO ₄ ³⁻	-	100/0.67/0.40	-

Note: SD refers to the standard deviation; ND = not detectable; detection limit = 1 mg/L. ¹ Based on Decree 883 [46]. BOD—biological oxygen demand, TCOD—total chemical oxygen demand, SCOD—soluble chemical oxygen demand, PCOD—particulate chemical oxygen demand, TKN—total Kjeldahl nitrogen, N-NH₄⁺—ammoniacal nitrogen, N—nitrogen, NO₂⁻—nitrite, NO₃⁻—nitrate, TN—total nitrogen, TP—total phosphorous, P-PO₄³⁻—orthophosphate, Cl⁻—chloride.

The average values of NTK and N-NH₄⁺ obtained from the effluent's physicochemical characterization were 13.1 and 8.5 mg/L, respectively. A low nitrogen content was observed, as vegetables such as pumpkin, cassava, and potato are primarily composed of water and carbohydrates, with moderate amounts of proteins and fats. Pumpkin, the main ingredient in the soups processed at the studied facility, contains 88.30 g of carbohydrates, 0.90 g of proteins, 9.50 g of fats, and 0.009 g of phosphorus per 100 g of raw vegetable.

Regarding total phosphorus and soluble phosphorus contents in the form of orthophosphate (P-PO₄³⁻), the average values were 6.3 and 4.8 mg/L, respectively. The presence of phosphorus is primarily attributed to the detergents used in cleaning machinery and production lines, as the contribution from the processed vegetables is negligible. Due to the nitrogen and phosphorus deficits, the addition of macronutrients is justified as an operational strategy to enhance the performance of the sequencing batch reactor (SBR) in organic matter removal.

The effluent had an average pH of 5.48, indicating an acidic character. Leifeld et al. [47] noted that food industry wastewater contains easily hydrolysable carbohydrates, which promote natural fermentation and the formation of volatile fatty acids, leading to low pH values. The food industry effluent also showed average turbidity values of 83 NTU and a color of 113 UC. The color of the wastewater is associated with the presence of vegetable pigments, influenced by chlorophylls, carotenoids, and anthocyanins, which give rise to green, red-yellow, and blue-violet hues, respectively.

During the characterization period, significant variability in the effluent's physicochemical characteristics was observed, as reflected by the high standard deviation values. This variability can be attributed to the seasonal nature of food processing campaigns, resulting in intermittent discharges with fluctuating daily characteristics due to discontinuous production processes and periodic cleaning operations.

The Venezuelan regulations establish specific parameters for the discharge of wastewater, distinguishing between discharges into natural water bodies (such as rivers, lakes, and seas) and discharges into public sewer systems (sewers). For discharges into natural water bodies, the permissible limits are stricter as they aim to protect water quality and minimize environmental impacts [47]. These parameters include maximum concentrations of pollutants such as the chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSSs), fats and oils, heavy metals, and other toxic compounds. In the case of discharges into sewer systems, the limits are generally less stringent since the wastewater will subsequently be treated in centralized treatment plants.

3.2. Biomass Adaptation Process to the Effluent

The microbial biomass was obtained from an aerobic treatment system located in a domestic liquid waste treatment plant. This biomass underwent an adaptation process using a batch reactor under aerobic conditions with 24-h cycles. During the adaptation process, the pH was maintained between 6.5 and 8.5, providing optimal conditions for the growth and reproduction of microorganisms [48].

Figure 3 shows that as the experimental time increased in the batch reactor, the COD removal percentage progressively improved. The fluctuation in COD concentration from day 1 to day 13 was primarily attributed to the “lag” phase. This phase occurs due to the physiological adjustment of cells to a new environment, which is commonly observed in industrial wastewater treatments. The acclimation period was crucial to gradually expose the microbial community to toxic or inhibitory organic compounds that may emerge over time as they adapt to the new conditions [49,50].

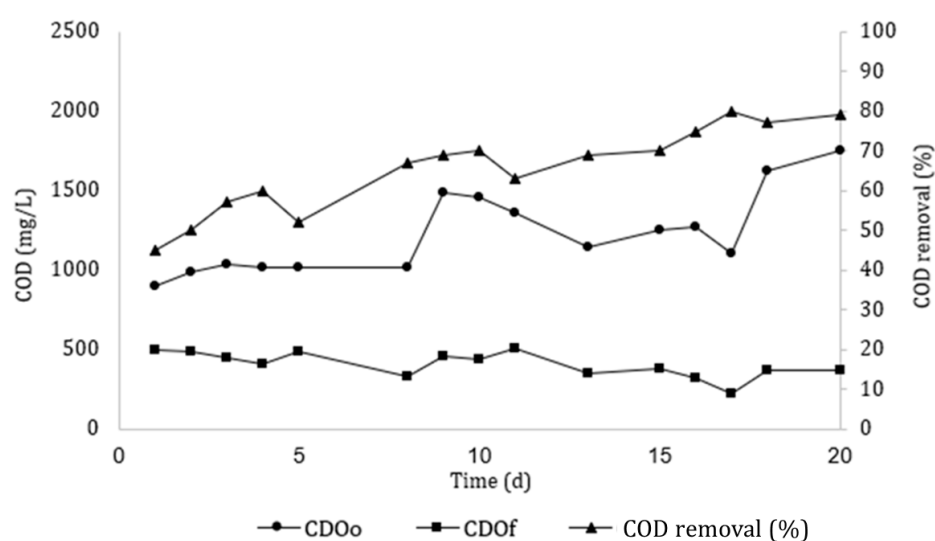


Figure 3. Biomass acclimatization process to the vegetable processing effluent. $[\text{COD}]_o$ represents the initial concentration of total COD (mg/L) and $[\text{COD}]_f$ represents the final concentration of total COD (mg/L).

An average removal efficiency of 52.8% was achieved during the first five days of reactor operation. Between days 6 and 10, an average removal of 68.6% was obtained, like the result between days 11 and 15, with 67.3%. However, between days 16 and 20, the reactor efficiency increased to 77.8%, while the outlet values stabilized around 320 mg/L. The adaptation process was considered complete after 20 days of operation, as high TCOD removal percentages (greater than 75%), good sludge sedimentation, and stable TCOD values at the reactor outlet were achieved.

At the end of the acclimation period, the reactor operated under stable and controlled conditions, demonstrating its efficiency in wastewater treatment. The operational parameters were as follows: an SV30 of 370 ± 20 mL/L, indicating an adequate sludge settling capacity, and an SVI30 of 107 ± 3 mL/g, confirming good sludge compaction and quality with a low risk of bulking. The concentration of total suspended solids in the mixed liquor (SSTML) was 3502 ± 128 mg/L, while the volatile suspended solid (VSSML) concentration reached 2515 ± 185 mg/L, representing a robust biomass with a predominant organic fraction. These results highlight the system's ability to maintain an optimal balance between sludge settleability and biological activity, ensuring an efficient biodegradation process of the organic matter in the influent.

3.3. Organic Matter Fractionation

The fractions of organic matter in the vegetable processing effluent for canned soup production are shown in Figure 4. The classification of the COD components was obtained according to their degradation rate and their soluble or particulate characteristics. The relevance of performing this fractionation lies in its ability to provide detailed information about the composition of the organic load, enabling the design and optimization of biological treatment processes. For instance, identifying the biodegradable fraction helps evaluate the efficiency of aerobic or anaerobic degradation processes, while the non-biodegradable fraction allows an estimation of the accumulation of matter in the system.

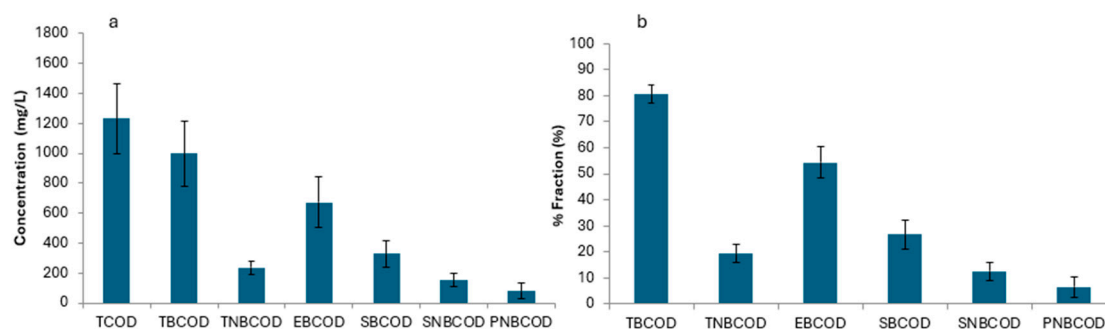


Figure 4. Comparison between (a) the concentrations and (b) the percentages of the different organic matter fractions in the vegetable processing effluent (the vertical bars represent the standard error. TCOD—total chemical oxygen demand; TBCOD—total biodegradable COD; TNBCOD—total non-biodegradable COD; EBCOD—easily biodegradable COD; SBCOD—slowly biodegradable COD; SNBCOD—soluble non-biodegradable COD; PNBCOD—particulate non-biodegradable COD.

The average COD of the effluent was 1232 mg/L, of which 80.6% corresponded to total biodegradable COD and 19.4% to non-biodegradable COD. The conventional biodegradability index, which is based on the BOD/COD ratio (0.68), was found to be lower than the biodegradable organic matter content (total biodegradable COD) obtained from the organic matter fractionation test. These results demonstrate that conventional characterization, which determines parameters such as COD and BOD in wastewater, does not provide accurate information about the organic matter removal efficiencies that could be achieved in biological systems.

The difference between the COD and total biodegradable COD highlights the importance of understanding COD fractions to properly design biological treatment systems. The design should be based on the biodegradable organic matter content rather than the total organic matter content [51,52]. Similar results were obtained by Xu et al. [53] when treating effluents from tomato processing, where they found a biodegradability index of 0.38, while COD fractionation showed that nearly 95% of the organic matter was biodegradable.

The total biodegradable COD results obtained (77.3–83.9%) were within the range reported (76.6–95.7%) for vegetable processing effluents [53,54]. Effluents from this type of industry are primarily composed of starch and soluble carbohydrates, which are assimilated by microorganisms at different rates [55]. These results are also like those obtained by Vavilin et al. [56] for domestic and food processing industry effluents, with total biodegradable CODs of 80% and 82%, and non-biodegradable CODs of 20% and 18%, respectively.

From the detailed fractionation of total biodegradable COD, it was found that 54.3% was readily biodegradable COD and 23.7% was slowly biodegradable COD. The easily biodegradable COD fraction consists of organic carbon compounds that serve as an energy source for microorganisms. However, these compounds must first undergo hydrolysis before they can diffuse and be absorbed by microorganisms for utilization [57,58]. This

fraction is consumed by microorganisms in the reactor and is not detected at the treatment outlet. For dairy wastewater, this fraction is dominant and ranges from 38.3% to 62.6% [59].

For non-biodegradable COD, 12.5% was soluble non-biodegradable COD, representing the proportion of organic matter that cannot be removed through biological methods, and the remaining 6.5% was particulate non-biodegradable COD. The value of readily biodegradable COD obtained was lower than that reported for wastewater from the poultry industry, where percentages ranged between 64.1% and 67.1% [60]. Knowing the readily biodegradable COD content in industrial wastewater is important because this type of organic matter is required by microorganisms in biological nitrogen removal processes.

A Pearson correlation matrix was generated (Table 5) to clarify the relationship between the COD of vegetable processing effluent and its fractions, revealing a strong positive correlation between the total biodegradable COD and COD ($r = 0.984$).

Table 5. Pearson’s correlation matrix for COD components in vegetable processing effluent.

	TBCOD	TNBCOD	EBCOD	SBCOD	SNBCOD	PNBCOD	TCOD
TNBCOD	0.359						
p	0.279						
EBCOD	0.913 **	0.351					
p	0.001	0.29					
SBCOD	0.635 *	0.139	0.272				
p	0.036	0.684	0.418				
SNBCOD	−0.005	0.283	−0.364	0.650 *			
p	0.989	0.4	0.271	0.031			
PNBCOD	0.391	0.713 *	0.611 *	−0.29	−0.427		
p	0.253	0.014	0.046	0.388	0.19		
TCOD	0.984 **	0.518	0.903 **	0.608 *	0.049	0.493	
p	0.001	0.103	0.001	0.047	0.887	0.123	
SCOD	0.582	0.486	0.591	0.244	0.134	0.352	0.625 *
p	0.061	0.13	0.056	0.469	0.694	0.288	0.04

* The correlation is significant at the 0.01 level. ** The correlation is significant at the 0.05 level. TBCOD—total biodegradable COD; EBCOD—easily biodegradable COD; SBCOD—slowly biodegradable COD; SNBCOD—soluble non-biodegradable COD; PNBCOD—particulate non-biodegradable COD.

This means that as the total COD of the effluent increases, so does the total biodegradable COD.

The fractionation provided insights into some of the operational conditions of the biological system, as the food effluent presented a low content of particulate non-biodegradable COD. Therefore, an intermediate to high SRT could be used [61]. For this reason, 15 and 25 days were evaluated as operational strategies during the treatment of effluents in the sequencing batch reactor.

From the fractionation, it was determined that 80.6% of the total COD could be removed by microorganisms in a biological treatment, and the concentration of soluble inert organic matter was 152 mg/L. Therefore, treatment in the sequencing batch reactor can produce an effluent that meets Venezuelan discharge regulations (350 mg/L) as the sole treatment for these effluents.

To complement the results of the biological treatability of the vegetable processing effluent, the determination of the biokinetic constants governing the biological treatment process was carried out. These constants will be used in the modeling and design of the purification system. The constants determined were the heterotrophic microorganism production rate (Y_h), the endogenous decay coefficient (K_d), and the substrate utilization rate (K).

The method described by Park et al. [38] was used in the experiment, where the effluent with the adapted biomass was placed in a batch reactor for 24 h, with a continuous air

supply and agitation. Figure 5 shows the evolution of substrate and biomass concentrations during one of the repetitions of the batch test. It can be observed that the concentrations of total COD and soluble COD decreased over the operational cycle due to oxidation caused by metabolic reactions and biomass cell growth, which consumed the organic matter, storing it within their cells or transforming it into final products such as CO_2 and water.

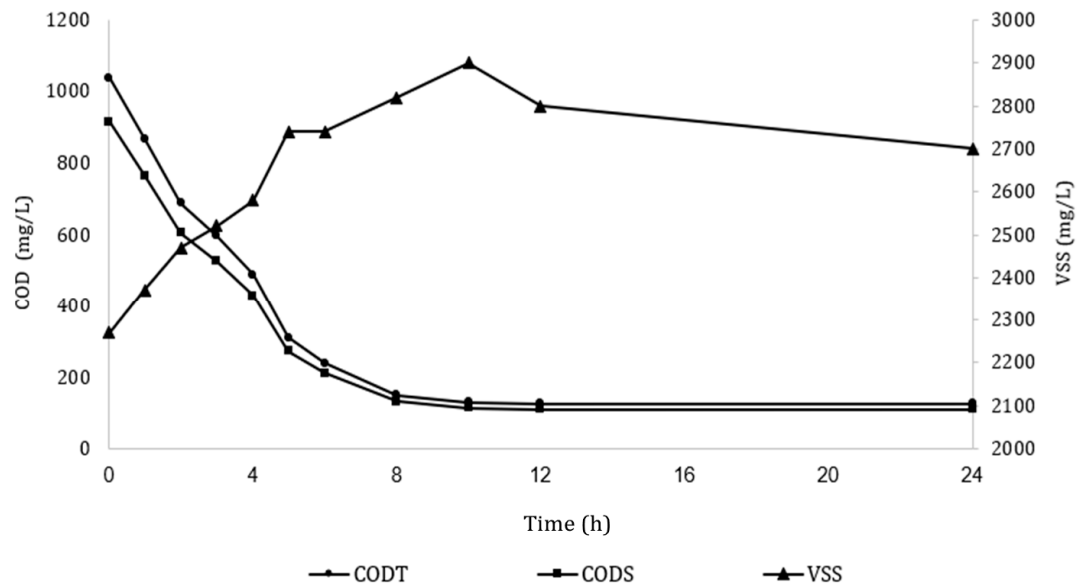


Figure 5. The evolution of the substrate concentration was measured as total COD (TCOD) and soluble COD (SCOD), and biomass was measured as volatile suspended solids (VSSs) during one repetition of the batch test for the determination of the Y_h and K_d constants.

The experimental COD and VSS data, taken from the initial portion of the curve where the biomass is in the logarithmic growth phase, were used to calculate the substrate utilization rate (U) and specific growth rate (μ) for each time point, with the results shown in Figure 6a. The average endogenous decay coefficient (K_d) from the seven repetitions performed was $0.113 \pm 0.086 \text{ d}^{-1}$ at 28.4°C . This value is lower than those reported by Durruty et al. [53] at 0.466 d^{-1} , Contreras et al. [62] at 0.160 d^{-1} , and Xu et al. [53] ranging from 0.150 to 0.240 d^{-1} .

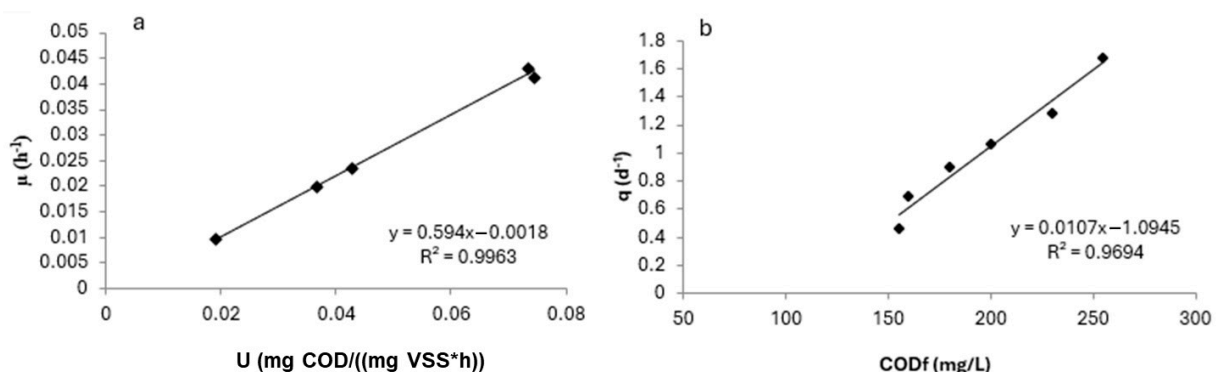


Figure 6. (a) Determination of the biokinetic constants Y_h and K_d . (b) Determination of the biokinetic constant K in the effluents from vegetable processing.

The heterotrophic cell production coefficient (Y_h) ranged from 0.510 to $0.758 \text{ mg VSS/mg COD}$, with an average value of $0.634 \pm 0.124 \text{ mg VSS/mg COD}$. These results are close to those reported by Xu et al. [52] of 0.760 for fermented effluents from tomato processing, and by Contreras et al. [62] and Durruty et al. [54] of 0.710 and $0.616 \text{ mg VSS/mg COD}$ for

potato processing effluents, respectively. This is also like the value reported for domestic wastewater, which is 0.67 mg VSS/mg COD [63]. The production coefficient is crucial for determining biomass production in biological systems, enabling the more accurate design of sludge treatment units [64].

Figure 6b shows the adjustment made to determine the substrate utilization rate (K) in one of the repetitions. K represents the volume (L) required per unit of COD mass consumed (mg) per day. It is expected that the points in the figure do not form a straight line with an R^2 correlation of 1, as sequencing batch reactors do not reach a steady state but rather a pseudo-steady state due to the nature of the process and the variability in the organic load concentration in the feed [65].

Following the organic matter fractionation process and the determination of biokinetic constants, the phase of applying operational strategies in the treatment of vegetable processing effluents began. The first strategy implemented was the addition of macronutrients (N and P) to improve the $\text{COD}/\text{N-NH}_4^+/\text{P-PO}_4^{3-}$ ratio and compensate for the deficiency of these compounds. The performance of two sequencing batch reactors was compared: one operating without nutrient addition (T1) and the other with macronutrient addition (T2).

3.4. Effect of Macronutrient Addition on Effluent Treatability

Following the organic matter fractionation process and the determination of biokinetic constants, the phase of applying operational strategies in the treatment of vegetable processing effluents began. The first strategy implemented was the addition of macronutrients (N and P) to improve the $\text{COD}/\text{N-NH}_4^+/\text{P-PO}_4^{3-}$ ratio and compensate for the deficit of these compounds, comparing the performance of two sequencing batch reactors: one operating without nutrient addition (T1) and the other with macronutrient addition (T2).

In Table 6, the average values of COD, soluble COD (SCOD), and BOD concentrations at the inlet and outlet of the sequencing batch reactor, as well as the removal percentages for both treatments applied, are shown. COD removal values of 79.6% and 78.3% were obtained for treatments without and with the addition of macronutrients, respectively, with no statistically significant differences ($p > 0.05$) found between these removal percentages. These results indicate that the addition of macronutrients did not improve the reactor's performance in removing organic matter, as measured by the COD. Similar results were obtained for soluble organic matter, for which no significant differences ($p > 0.05$) were found in the SCOD removal percentages.

Table 6. Concentrations of organic matter measured as TCOD, SCOD, and BOD at the input and output of the treatment of vegetable processing effluents with and without the addition of macronutrients.

Variable (mg/L)	Phase	T1 (Mean $\pm$ SD)	T2 (Mean $\pm$ SD)
TCOD	Input	1158 $\pm$ 160	955 $\pm$ 181
	Output	232 $\pm$ 31.0	265 $\pm$ 54.0
SCOD	Input	764 $\pm$ 95.0	626 $\pm$ 122
	Output	174 $\pm$ 24.0	149 $\pm$ 42.0
BOD	Input	719 $\pm$ 160	764 $\pm$ 146
	Output	66 $\pm$ 14.0	84 $\pm$ 27
TCOD Removal (%)		79.6 $\pm$ 3.50 ^a	78.7 $\pm$ 6.30 ^a
SCOD Removal (%)		77.3 $\pm$ 1.20 ^a	76.3 $\pm$ 6.70 ^a
BOD Removal (%)		90.5 $\pm$ 2.60 ^a	88.9 $\pm$ 3.30 ^a

Note: SD—standard deviation; n = 12, n—number of repetitions; T1—treatment without the addition of macronutrients (N and P); T2—treatment with the addition of macronutrients. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$). BOD—biological oxygen demand, TCOD—total chemical oxygen demand, SCOD—soluble chemical oxygen demand.

The values of TCOD at the outlet of the sequencing batch reactor (SBR) ranged between 201 and 319 mg/L, complying with Venezuelan regulations for discharge into water bodies in both treatments [47]. On the other hand, the values of SCOD at the reactor outlet, corresponding to the inert soluble fraction that cannot be removed by biological processes, ranged between 107 and 198 mg/L. These values represented 15.0% and 15.6% of the total organic matter influent to the reactor, which were close to the concentration of inert biodegradable soluble organic matter obtained from the COD fractionation (12.5%).

Regarding the organic matter measured as biochemical oxygen demand (BOD), the removal percentages ranged between 85.6% and 93.1%, with average values of 90.5% for T1 and 88.9% for T2. As with TCOD and SCOD, no significant differences ($p > 0.05$) were found in the BOD removal percentages between the two treatments, indicating that the addition of macronutrients as an operational strategy did not improve the removal of biodegradable organic matter.

These results suggest that the biomass present in the reactor could remove organic matter from the wastewater despite the nutritional limitations. This finding is consistent with the reports of Gurtekin [66], Kargi and Uygun [67], Roy et al. [68], who successfully applied biological treatments to effluents with nitrogen and phosphorus deficiencies. Similarly, Xu et al. [52] successfully operated a biological treatment system for tomato processing effluents with an ammoniacal nitrogen deficiency, achieving a 99% removal percentage for BOD and settleable solids.

The average BOD values at the reactor outlet were 66 mg/L and 84 mg/L for T1 and T2, respectively, which are slightly higher than the limits established in Venezuelan regulations for discharges into water bodies [47]. Therefore, operational strategies such as increasing the cell retention time and cycle operational time were applied in the subsequent stages of the research.

The concentrations of nitrogen compounds at the inlet and outlet of the two treatments are presented in Table 7. For treatment T1, the concentrations of NTK and $\text{NH}_4^+\text{-N}$ were 10.0 and 7.5 mg/L, respectively, while for treatment T2, the concentrations were doubled to 20.8 and 15.6 mg/L due to the addition of urea.

Table 7. Concentrations of nitrogen forms at the input and output of the treatment of vegetable processing effluents with and without the addition of macronutrients.

Variable (mg/L)	Phase	T1 (Mean $\pm$ SD)	T2 (Mean $\pm$ SD)
TKK	Input	10.0 $\pm$ 1.5	20.8 $\pm$ 2.2
	Output	7.5 $\pm$ 0.9	8.5 $\pm$ 5.1
N- NH_4^+	Input	7.5 $\pm$ 1.1	15.6 $\pm$ 3.03
	Output	5.4 $\pm$ 0.6	5.6 $\pm$ 3.4
N- NO_2^-	Input	ND	ND
	Output	ND	0.84 $\pm$ 0.57
N- NO_3^-	Input	ND	ND
	Output	ND	5.50 $\pm$ 3.10
NTK removal (%)		23.0 $\pm$ 15.6 ^b	60.2 $\pm$ 21.9 ^a
N- NH_4^+ removal (%)		25.2 $\pm$ 15.7 ^b	63.3 $\pm$ 21.4 ^a

TKN—total Kjeldahl nitrogen, N- NH_4^+ —ammoniacal nitrogen, N—nitrogen, N- NO_2^- —nitrite, N- NO_3^- —nitrate. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

From the analysis of variance and mean separation, significant differences ($p \leq 0.05$) were found between the NTK and $\text{NH}_4^+\text{-N}$ removal percentages between the two treatments. This is because nitrogen removal by both treatments occurred through different

biological processes. The two main biological mechanisms involved in nitrogen removal are assimilation and nitrification–denitrification. Similarly, ammoniacal nitrogen can also be removed from effluents through abiotic processes, such as volatilization.

For the treatment without nutrient addition (T1), it is inferred that nitrogen removal occurred primarily via assimilation, as no nitrates or nitrites were detected at the end of the operational cycle, which was entirely aerobic. According to Garzón [69], microorganisms in wastewater tend to assimilate ammoniacal nitrogen and incorporate it into their cell mass. Therefore, the reactor likely underwent the ammonification of organic nitrogen present in the industrial effluent, followed by its subsequent assimilation by the biomass. Pire et al. [70] and Lefebvre et al. [71] pointed out that low NTK removal percentages indicate that nitrogen elimination occurred solely through assimilation, and not through nitrification–denitrification processes.

On the contrary, it is inferred that the ammoniacal nitrogen removal process that prevailed in treatment T2, with nutrient addition, was nitrification. This is supported by the detection of nitrite and nitrate concentrations at the reactor outlet, with average values of 0.84 mg/L and 5.50 mg/L, respectively. Urea is highly soluble in water, hydrolyzes rapidly, and upon the action of urease, produces ammonium carbonate. This salt dissociates to form ammoniacal nitrogen and carbonates. The ammoniacal nitrogen is then transformed into nitrite and subsequently nitrate by nitrifying bacteria. However, a complete nitrification process was not achieved, as ammoniacal nitrogen and NTK were still present at the reactor outlet, with average values of 5.6 mg/L and 8.5 mg/L, respectively.

The occurrence of incomplete nitrification may have been caused by the pH values at the inlet of the vegetable processing effluent, which ranged between 6.52 and 6.94. These values are outside the optimal range for nitrifying bacteria, which is between 7.5 and 8.6 [72]. Additionally, it was observed that during treatment T2, the alkalinity at the reactor inlet decreased, which may have also contributed to the incomplete nitrification process.

The addition of nitrogen and phosphorus in the sequencing batch reactor did not improve the removal of organic matter. No statistically significant differences ($p > 0.05$) were found between the percentages of TCOD, SCOD, and BOD removal between treatments with and without the addition of macronutrients. Therefore, this strategy was not used in the subsequent phases of the research. The reactor operated without nutrient addition achieved TCOD removal close to 80%, generating an effluent that complies with Venezuelan regulations. Moreover, considering that the effluent contained 19.4% inert organic matter, the percentage of biodegradable organic matter removal achieved by the treatment was 99.4%.

3.5. Performance of the SBR at Different OCTs and SRTs

The efficiency of organic matter and nutrient removal was evaluated in two sequencing batch reactors (SBR) operating in parallel and independently under different operational cycle times (OCTs) and sludge retention times (SRTs). Table 8 presents the results of the evaluation of the operational parameters in the SBR.

The operating temperature of the SBRs for the six treatments applied was similar, ranging between 27.5 and 29.2 °C, indicating a mesophilic working range [73]. This temperature range falls within the values recommended for optimal biological activity. As the system temperature increases, microbial activity also increases, particularly for nitrifying bacteria, which are highly sensitive to temperature variations.

However, differences were observed in the organic volumetric loading rate (OVL) due to the inherent variability of the effluent, as previously noted in the characterization, and because the operational cycle time (OCT) varied in the applied treatments. OVL values ranged between 1.93 kg/m³·day and 3.59 kg/m³·day, which were higher than

those reported by Díaz et al. [74], who successfully treated shrimp industry effluents in a sequential batch reactor with an average OVL of 1.27 kg/m³·d.

Table 8. Operational parameters in the SBR for the treatments evaluated in the vegetable processing effluent.

Tr	pH		T (°C)	OVL	MLSST	MLVSS	DO
	Input	Output		Kg COD/(m ³ ·day)	(mg/L)	(mg/L)	(mg/L)
T1	7.37 ± 0.19	8.32 ± 0.12	27.5 ± 0.5	3.59 ^a ± 0.51	3340 ± 590	2270 ± 480	3.1 ± 0.5
T2	7.31 ± 0.56	8.00 ± 0.39	28.5 ± 0.9	2.16 ^c ± 0.06	3630 ± 184	2930 ± 184	3.5 ± 0.8
T3	7.58 ± 0.10	8.12 ± 0.47	29.2 ± 0.7	1.94 ^{c,d} ± 0.15	3930 ± 387	2750 ± 332	3.2 ± 0.4
T4	7.02 ± 0.08	6.73 ± 0.10	28.2 ± 0.3	2.50 ^b ± 0.14	3813 ± 580	2740 ± 258	3.7 ± 0.3
T5	7.57 ± 0.15	6.60 ± 0.12	28.5 ± 0.3	2.15 ^c ± 0.10	3630 ± 484	2670 ± 221	3.5 ± 0.2
T6	7.08 ± 0.18	6.63 ± 0.15	28.7 ± 0.7	1.83 ^d ± 0.11	2940 ± 532	2600 ± 201	3.1 ± 0.9

Note: Values are presented as means ± SDs. SD—standard deviation; Tr—treatment; n—number of repetitions, n = 12; T1—treatment with an OCT of 6 h and an SRT of 15 days; T2—8 h and 15 days; T3—10 h and 15 days; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; OVL—organic volumetric loading rate; DO—dissolved oxygen; MLVSSs—volatile suspended solids in mixed liquor. Means followed by different letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

The concentration of MLTSSs was found to be between 2940 and 3930 mg/L, while the average values of MLVSS ranged from 2270 to 2670 mg/L. According to Budiastuthe et al. [75], the typical concentration of suspended solids in mixed liquor should range between 2000 and 4000 mg/L, values close to those obtained for the applied treatments, indicating good system performance.

On average, the MLVSS/LMTSS ratios for reactors operated with SRTs of 15 and 25 days were 0.72 and 0.78, respectively. These values are within the recommended range by Tewari et al. [76], who suggested that the MLVSS/LMTSS ratio should be maintained between 0.7 and 0.9 during biological treatment to avoid sludge bulking and ensure good sedimentation. Valderrama et al. [77] evaluated the biological treatment of effluents from the wine production industry, reporting an MLVSS/LMTSS ratio of 0.75, which did not present operational issues such as sludge bulking or rising.

The efficiency values of the SBRs for the removal of total COD are shown in Table 9. It was observed that the removal percentages ranged between 75.9% and 84.8%, with the highest removal achieved in treatments with an OCT of 8 h (T2 and T5) and 10 h (T3 and T6), regardless of the SRT used ($p > 0.05$). No significant differences ($p > 0.05$) were found between treatments T1 and T4, which had the same OCT but different SRTs, indicating that the variation in SRT did not affect TCOD removal.

The average organic matter removal rate ranged between 90.5 and 167.5 mg COD/(L·h), being higher during treatment T1. This can be attributed to the higher initial concentration of organic matter in the effluent and the shorter operational cycle time (OCT) of 6 h. When comparing rates with the same OCT but different sludge retention times (SRTs), no significant differences ($p > 0.05$) were found, indicating that increasing the SRT did not affect the organic matter removal rate. The highest specific removal rate (0.074 mg COD/(mg VSS·h)) was obtained for treatment T1, while for the other treatments, the specific removal rate ranged between 0.035 and 0.074 mg COD/(mg VSS·h), with no significant differences ($p > 0.05$).

Figure 7 presents the TCOD profiles throughout the operational cycle time (TCO) in the SBR, generally showing a decrease in the TCOD concentration across the reactor's operational cycle. By the end of the filling stage of each treatment, an average reduction of 14.3% was achieved. During this initial stage, which lasted only 15 min of static filling, organic compounds were absorbed into the activated sludge. Microorganisms absorbed most of the dissolved organic matter for processes such as energy production and the

synthesis of new cells and cellular material. In the subsequent aerobic reaction stage, the removal percentage increased as metabolic oxidation and assimilation occurred to eliminate EBCOD (biologically degradable COD). This phenomenon is referred to as assimilative oxidation. The results indicated that increasing the hydraulic retention time (HRT) improved the performance of both the SBR and BSBR systems, particularly in terms of COD removal, with the BSBR showing superior results. This improvement in reactor performance can be attributed to the extended interaction between microorganisms and organic matter, which enhances the removal of COD from the influent [78].

Table 9. Efficiency of COD removal for the treatments evaluated in the effluent from vegetable processing.

Treatment	TCOD (mg/L)			% COD Removal (%)	Organic Matter Removal Rate (mg COD/(L.h))	Specific Organic Matter Removal Rate
	Inlet	End of Filling	Outlet			(mg COD/mg VSS.h)
T1	1283 ± 83	898 ± 178	278 ± 60	77.9 ± 5.7 ^{b,c}	167.5 ± 32.7 ^a	0.074 ± 0.015 ^a
T2	1028 ± 29	999 ± 28	171 ± 54	83.4 ± 5.3 ^{a,b}	107.2 ± 7.9 ^{b,c}	0.037 ± 0.003 ^b
T3	1156 ± 88	1077 ± 82	174 ± 53	84.8 ± 4.9 ^a	98.2 ± 10.6 ^{b,c}	0.036 ± 0.004 ^b
T4	894 ± 50	831 ± 65	217 ± 22	75.9 ± 6.4 ^c	112.8 ± 9.3 ^b	0.041 ± 0.004 ^b
T5	1025 ± 50	953 ± 78	173 ± 52	83.2 ± 5.1 ^{a,b}	106.5 ± 8.1 ^{b,c}	0.040 ± 0.003 ^b
T6	1087 ± 67	1010 ± 51	184 ± 35	83.1 ± 4.7 ^{a,b}	90.6 ± 7.9 ^c	0.035 ± 0.003 ^b

Note: n—number of repetitions, n = 12; T1—treatment with a operational cycle time (TCO) of 6 h and a sludge retention time (SRT) of 15 days; T2—8 h and 15 days; T3—10 h and 15 days; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; SRT—sludge retention time; TCO—total chemical oxygen demand. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

It was observed that during the first three hours of the operational cycle, the highest organic matter removal occurred, with average values of 52.8%, 54.2%, and 54.0% for the 6-h, 8-h, and 10-h treatments, respectively. This is because vegetable processing effluents contain high concentrations of easily degradable soluble organic compounds, which can be directly adsorbed by the cell walls of microorganisms and metabolized without the need for hydrolysis. Hydrolysis is typically the limiting step in the removal of organic compounds during biological treatment. The hydrolysis of organic compounds is a critical step that involves the decomposition of macromolecular organic compounds (such as carbohydrates, proteins, and lipids) into simpler, soluble molecules through the action of hydrolytic enzymes produced by microorganisms. Bacteria secrete extracellular enzymes, such as amylases (for carbohydrates), proteases (for proteins), and lipases (for lipids), which break down complex organic matter into smaller units: carbohydrates into monosaccharides, proteins into amino acids, and lipids into fatty acids and glycerol.

For the treatments with a 10-h operational cycle (TCO), it was observed that after the eighth hour, the curves reached a point of stability, with a residual DQOT concentration of 167 mg/L remaining. This is because the organic matter in vegetable processing effluents consists of fractions that may not be degraded by microorganisms, leaving behind inert or refractory fractions [51]. This remaining DQOT concentration represented approximately 15.1% of the total organic matter in the effluent, a value close to the DQONBs reported by Park et al. [38].

The COD profiles conducted for each treatment show very similar organic matter degradation kinetics. The degradation kinetics followed a pseudo-first-order reaction and could be represented by Equation (5), where $-dC/dt$ is the rate of organic matter degradation (mg/(L.h)), C is the organic matter concentration (mg/L), and K_b is the biodegradation constant (h^{-1}):

$$-dC/dt = K_b \times C \quad (5)$$

By integrating Equation (5) and rearranging the terms, the linear form shown in Equation (6) is obtained, whose graphical representation is presented in Figure 8.

$$\ln C = -K_b \times t + \ln C_0 \quad (6)$$

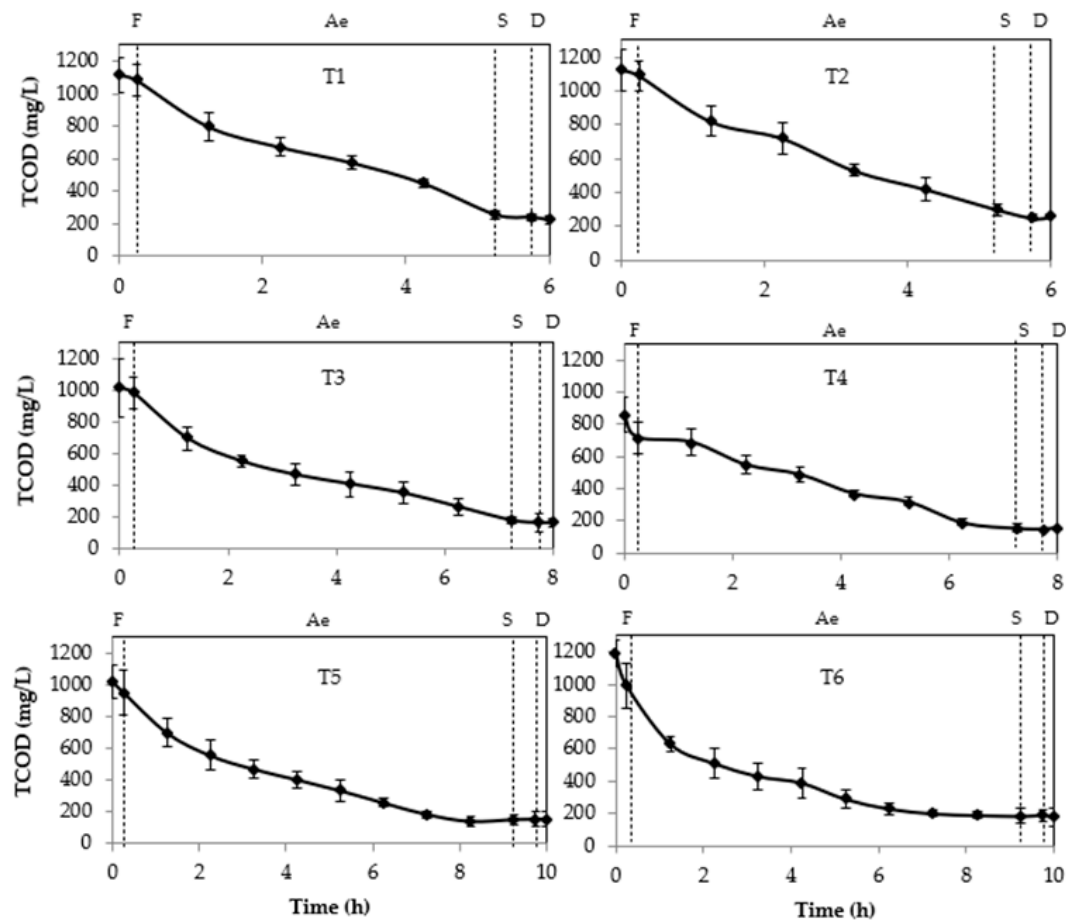


Figure 7. Evolution of TCOD during the treatments applied to the vegetable processing effluent. (The vertical bars represent the standard error. T1—treatment with an OCT of 6 h and an SRT of 15 days; T2—8 h and 15 days; T3—10 h and 15 days; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; LI—filling; Ae—aerobic reaction phase; S—sedimentation; D—discharge; OCT—operational cycle time; SRT—sludge retention time).

It was observed that for the total COD (TCOD), as the concentration at the inlet increased, lower concentrations were found at the outlet, leading to higher removal percentages. This behavior was also reported by Carrasquero et al. [26] during the treatment of tannery effluent in a sequencing batch reactor (SBR) operated with a 12-h cycle. From the Pearson correlation analysis between the organic volumetric loading rate (OVL) and the removed COD ($p \leq 0.05$), it was found that, for all evaluated operational cycle times (OCTs), as the COV of the effluent increased, the percentage of TCOD removal also increased (Figure 9). Similarly, a significant correlation ($p \leq 0.05$) was found between the OVL and the rate of organic matter removal (Figure 10), with a positive correlation coefficient of 0.955.

The removal of organic matter measured as BOD reached values ranging from 87.0 to 92.0%, with average values exceeding 90% when OCTs greater than 6 h were used (Table 10). Statistically significant differences ($p \leq 0.05$) were found between the values obtained for the treatment with a 6-h OCT and the other two treatments with 8 and 10 h, indicating that the removal percentage also increased as the OCT increases. However, no significant differences ($p > 0.05$) were found between the values obtained for the treatments with 8- and 10-h OCTs, suggesting that the optimal OCT for the removal of TCOD and BOD was

8 h. When comparing treatments with the same OCT but different SRTs, no significant differences ($p > 0.05$) were found in all cases evaluated, indicating that varying the SRT did not affect BOD removal.

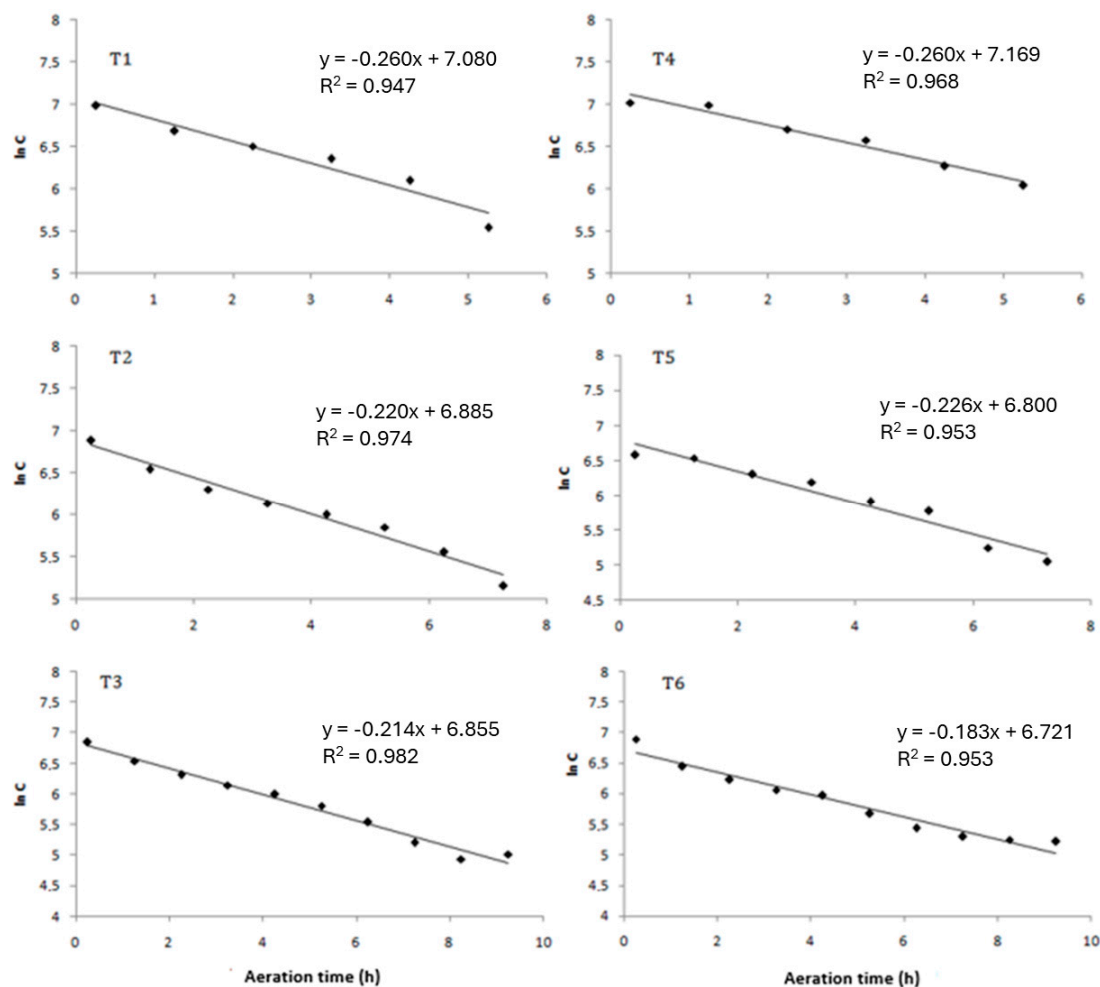


Figure 8. Pseudo-first-order degradation kinetics. (T1—treatment with a cycle time (TCO) of 6 h and a cell retention time (TRC) of 15 days; T2—8 h and 15 days; T3—10 h and 15 days; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; TCO—operational cycle time; TRC: cell retention time).

Table 10. Efficiency of BOD removal for the treatments evaluated in the vegetable processing effluent.

Variable (mg/L)	Phase	T1	T2	T3	T4	T5	T6
BOD	Inlet	669 ± 203	649 ± 175	659 ± 155	754 ± 200	707 ± 142	683 ± 155
	End of filling	549 ± 165	506 ± 136	527 ± 124	603 ± 52	625 ± 90	560 ± 58
	Outlet	82 ± 14	49 ± 9	50 ± 14	81 ± 28	48 ± 10	55 ± 8
BOD removal (%)		87.0 ± 3.2 ^c	92.0 ± 2.3 ^{a,b}	91.9 ± 3.1 ^{a,b}	88.6 ± 3.1 ^{b,c}	92.19 ± 2.0 ^a	91.7 ± 1.7 ^{a,b}

Note: T1—treatment with a 6-h OCT and a 15-day SRT; T2—8-h OCT and 15-day SRT; T3—10-h OCT and 15-day SRT; T4—6-h OCT and 25-day SRT; T5—8-h OCT and 25-day SRT; T6—10-h OCT and 25-day SRT; BOD—biological oxygen demand. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

The nitrogen removal efficiency values are shown in Table 11. The removal of TKN ranged between 21.9% and 64.6%, with removal percentages exceeding 55% when an SRT of 25 days was used. Significant differences ($p \leq 0.05$) were found between the treatments with an SRT of 15 days (T1, T2, and T3) and the treatments with 25 days (T4, T5, and T6), indicating that the variation in the SRT positively affected TKN removal.

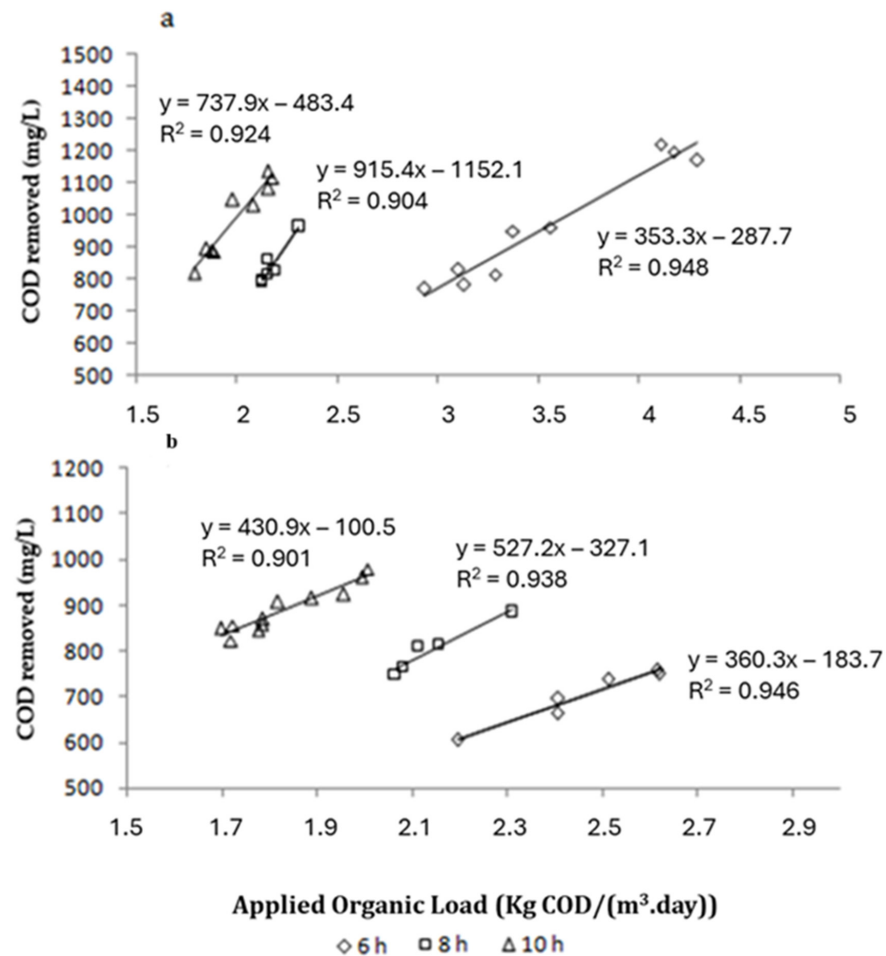


Figure 9. Relationship between the applied volumetric organic load and the COD removed during the effluent treatment from vegetable processing with an SRT of 15 days (a) and 25 days (b).

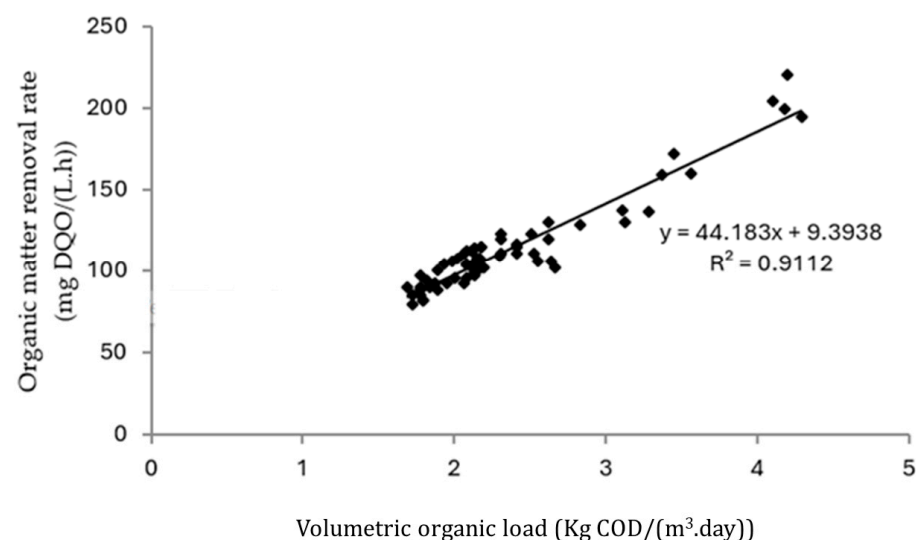


Figure 10. Relationship between the applied volumetric organic load and the organic matter removal rate during the treatments of the effluent from vegetable processing.

A similar behavior was observed for ammoniacal nitrogen. In treatments T4, T5, and T6, removal percentages of 55.1%, 61.5%, and 58.4% were obtained, respectively. This difference may be attributed to the occurrence of nitrification in the treatments with a TRC of 25 days, as average concentrations of nitrites plus nitrates of 2.60, 3.68, and 2.63 mg/L

were recorded at the end of the aerobic operational cycle in the reactors. In contrast, in the treatments with an SRT of 15 days, the removal of TKN and N-NH_4^+ occurred through assimilatory processes.

Table 11. Removal efficiencies of NT, N-NH_4^+ , and NTK for the treatments evaluated in the vegetable processing effluent.

Variable (mg/L)	Phase	T1	T2	T3	T4	T5	T6
TN	Inlet	11.5 ± 1.1	11.5 ± 0.4	11.4 ± 0.6	11.7 ± 1.1	11.9 ± 1.4	11.8 ± 1.2
	Outlet	8.3 ± 0.4	9.8 ± 0.8	8.4 ± 1.1	7.8 ± 0.2	7.9 ± 0.5	7.4 ± 0.7
TKN	Inlet	11.4 ± 1.1	11.5 ± 0.4	11.4 ± 0.6	11.7 ± 1.1	11.9 ± 1.4	11.8 ± 1.2
	Outlet	8.3 ± 0.4	9.0 ± 0.8	9.7 ± 0.4	5.2 ± 0.7	4.2 ± 0.5	4.8 ± 0.7
N-NH_4^+	Inlet	9.1 ± 0.7	10.3 ± 0.2	9.8 ± 0.8	9.8 ± 0.5	10.5 ± 0.3	9.2 ± 0.4
	Outlet	7.3 ± 0.6	8.0 ± 0.5	7.9 ± 0.3	4.4 ± 1.0	4.0 ± 1.0	3.8 ± 0.5
N-NO_x^-	Inlet	ND	ND	ND	ND	ND	ND
	Outlet	ND	ND	ND	2.60 ± 0.13	3.68 ± 0.65	2.63 ± 1.1
TKN removal (%)		28.0 ± 5.9 ^{c,d}	21.9 ± 5.4 ^d	26.3 ± 7.0 ^{c,d}	55.3 ± 2.3 ^b	64.6 ± 4.7 ^a	59.6 ± 6.4 ^a
N-NH_4^+ removal (%)		20.0 ± 6.7 ^c	22.1 ± 3.2 ^c	19.30 ± 4.8 ^c	55.1 ± 1.8 ^b	61.5 ± 3.0 ^a	58.4 ± 5.6 ^a
TN removal (%)		28.0 ± 5.9 ^{b,c}	21.9 ± 5.4 ^c	26.3 ± 7.0 ^{b,c}	33.1 ± 6.8 ^{a,b}	33.6 ± 4.7 ^{a,b}	37.2 ± 5.4 ^a

Note: n—number of repetitions, n = 12; T1—treatment with an OCT of 6 h and an SRT of 15 days; T2—8 h and 15 days; T3—10 h and 15 days; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; OCT—operational cycle time; SRT—sludge retention time; ND—not detectable; detection limit—1 mg/L; TKN—total Kjeldahl nitrogen; N-NH_4^+ —ammoniacal nitrogen; TN—total nitrogen; NO_x^- —nitrites + nitrates. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

A high sludge age is associated with large sludge masses in the reactor, which leads to large reactor volumes required. Therefore, even with solid separation, as the SRT increases, so does the HRT. The link between SRT and HRT is neither linear nor proportional and depends on (i) the concentration of organic matter in the wastewater (COD or BOD) and (ii) the concentration of total suspended solids. In systems with biological nutrient removal, the sludge age is around 10 to 25 days, while the nominal hydraulic retention time is on the order of 10 to 24 h [79]. The removal of nitrogen by assimilation is based on the uptake and incorporation of nitrogen compounds into microbial biomass during biological wastewater treatment processes. In this pathway, microorganisms, primarily bacteria, algae, and fungi, utilize nitrogen in the form of ammonium (NH_4^+), nitrate (NO_3^-), or organic nitrogen as a nutrient source for their growth and metabolic functions.

In Table 12, the results of total phosphorus measurements for each evaluated treatment are shown. Removal efficiencies ranged between 21.1% and 28.1%, with no statistically significant differences ($p > 0.05$) between the treatments, indicating that the variations in SRT and HRT did not affect phosphorus removal. Similar results were reported by Carrasquero et al. [80], who varied the SRT from 15 to 25 days during biological nutrient removal from tannery effluents and found that the variation in the solid retention time was not a factor influencing phosphorus removal, but it did affect nitrogen removal. Conversely, Akin and Urgulu [81] observed that the highest efficiency in terms of phosphorus removal was achieved at low solid retention times (10 days).

Due to the absence of an anaerobic–aerobic–anoxic sequence that allows for the proliferation of phosphorus-accumulating organisms, it is inferred that this nutrient is removed through assimilation. The removal of phosphorus by assimilation occurs when microorganisms incorporate phosphorus into their cellular biomass as an essential nutrient for growth and metabolism. Phosphorus, typically in the form of orthophosphate (PO_4^{3-}), is taken up by bacteria, algae, and other microorganisms and is utilized to synthesize key cellular com-

ponents such as nucleic acids (DNA and RNA), phospholipids, and adenosine triphosphate (ATP), which are critical for cellular function, energy transfer, and structural integrity.

Table 12. Efficiency of total phosphorus, color, and turbidity removal for the treatments evaluated in the effluent from vegetable processing.

Variable (mg/L)	Fase	T1	T2	T3	T4	T5	T6
TP	Inlet	8.68 ± 0.68	8.29 ± 0.40	5.01 ± 0.31	4.69 ± 0.52	4.88 ± 0.58	4.89 ± 0.44
	Outlet	6.61 ± 0.70	6.18 ± 0.24	3.61 ± 0.36	3.69 ± 0.32	3.81 ± 0.34	3.71 ± 0.24
Color (Pt-Co Units)	Inlet	167 ± 22	134 ± 32	155 ± 26	91 ± 13	113 ± 17	89 ± 21
	Outlet	47 ± 10	38 ± 15	43 ± 11	36 ± 13	35 ± 13	25 ± 9
Turbidity (NTU)	Inlet	122.0 ± 25.60	75.8 ± 3.9	96.8 ± 27.9	91.6 ± 21.3	88.1 ± 16.9	87.6 ± 17.6
	Outlet	19.4 ± 7.60	21.0 ± 4.24	31.4 ± 2.80	27.3 ± 2.80	18.5 ± 2.90	16.0 ± 3.40
TP removal (%)		23.6 ± 7.0 ^{a,b}	25.2 ± 5.1 ^{a,b}	28.1 ± 3.4 ^a	21.1 ± 6.5 ^b	21.8 ± 5.1 ^{a,b}	24.0 ± 3.8 ^{a,b}
Color removal (%)		71.6 ± 6.50 ^a	70.6 ± 11.9 ^a	72.3 ± 6.10 ^a	60.1 ± 15.0 ^a	68.6 ^a ± 11.2 ^a	70.5 ± 10.7 ^a
Turbidity removal (%)		83.3 ± 9.20 ^a	72.2 ± 5.90 ^{b,c,d}	64.4 ± 13.4 ^d	67.7 ± 9.80 ^{c,d}	78.3 ± 5.50 ^{a,b,c}	81.1 ± 6.10 ^{a,b}

TP—total phosphorous. Means followed by different superscript letters in each column indicate significant differences according to Tukey's test ($p \leq 0.05$).

The color and turbidity removal efficiencies for the sequencing batch reactors are shown in Table 13. Color removal percentages ranged from 60.1% to 72.3%, with no significant differences ($p > 0.05$) between the treatments performed. The color removal percentages were lower than those reported by He et al. [82], who, when treating effluents from a rice, flour, and vegetable processing industry, reported an average color removal efficiency of 93%. The color values at the inlet of the reactors ranged from 89 to 167 Pt-Co units. The color of industrial effluents depends on the substances and materials present. In the case of effluents from vegetable processing, the coloration may largely be due to the presence of carotenoids, which are responsible for most of the yellow, orange, or red colors found in plant-based foods.

Table 13. pH and alkalinity values at the inlet and outlet of the treatments evaluated in the effluent from vegetable processing.

Variable	Phase	T1	T2	T3	T4	T5	T6
pH	Inlet	7.37 ± 0.19	7.31 ± 0.56	7.58 ± 0.10	7.02 ± 0.08	6.57 ± 0.15	7.08 ± 0.18
	Outlet	8.32 ± 0.12	8.00 ± 0.39	8.12 ± 0.47	6.73 ± 0.10	6.60 ± 0.12	6.63 ± 0.15
Total alkalinity (mg CaCO ₃ /L)	Inlet	390 ± 83	204 ± 39	217 ± 61	211 ± 34	164 ± 16	189 ± 25
	Outlet	402 ± 159	208 ± 40	290 ± 99	171 ± 38	115 ± 24	143 ± 31

Note: n—number of repetitions, n = 12; T1—treatment with an OCT of 6 h and a CRT of 15 days; T2—8 h and 15 days; T3—10 h and 15 days.; T4—6 h and 25 days; T5—8 h and 25 days; T6—10 h and 25 days; OCT—operational cycle time; CRT—cellular retention time.

Color removal in wastewater through biological pathways occurs primarily via the action of microorganisms capable of degrading or transforming complex organic compounds responsible for color, such as dyes, lignins, and humic substances. This process involves the secretion of extracellular enzymes like laccase, lignin peroxidase, and manganese peroxidase, which break down chromophore structures (color-causing chemical bonds) into simpler, colorless molecules. Another mechanism involves the adsorption of color-causing compounds onto the surface of activated sludge or biofilms, where they are either metabolized or immobilized, reducing their concentrations in the effluent. The efficiency of biological color removal depends on the nature of the wastewater and the type of compounds present.

Turbidity removal percentages ranged from 64.4% to 83.3%. The significant differences observed ($p \leq 0.05$) between treatments could not be attributed to the modification of the

SRT or HRT, but rather to the high variability in turbidity at the start of treatments. The highest removal percentage (83.3%) was achieved in treatment T1, with the highest initial turbidity level (167 NTU).

Turbidity removal in biological treatment systems is primarily achieved through flocculation, adsorption, and sedimentation processes. Microorganisms in activated sludge systems aggregate to form bioflocs that trap fine suspended solids and colloidal particles. These flocs, which are denser and larger, settle during the sedimentation phase, resulting in reduced turbidity. Furthermore, the bacterial biomass can adsorb colloidal particles onto its surface, effectively removing them from the water column. The biodegradation of soluble organic matter that may later contribute to turbidity is another important mechanism.

The average pH and total alkalinity values at the inlet and outlet of the system for the evaluated treatments are presented in Table 13. Total alkalinity values ranged from 164 to 390 mg CaCO₃/L. This alkalinity provides the biological system with the capacity to neutralize acids produced by the oxidation of organic matter and ammonia.

For treatments T1, T2, and T3, an increase in pH was observed at the reactor outlet. The removal of the acidic gas (CO₂) decreased the concentration of hydronium ions [H⁺], consequently increasing the pH at the reactor outlet. The CO₂ produced during the decomposition of organic matter combines with water to form carbonic acid (H₂CO₃), which can dissociate into bicarbonate, increasing alkalinity. The Pearson correlation analysis revealed a significant correlation ($p \leq 0.05$) between the COD and the total alkalinity of the effluent from vegetable processing, with a positive correlation coefficient ($r = 0.807$).

From the Pearson correlation analysis, a significant correlation ($p \leq 0.05$) was obtained between the COD and the total alkalinity of the effluent from vegetable processing, with a positive correlation coefficient ($r = 0.807$).

On the contrary, for treatments T4, T5, and T6, decreases in pH and alkalinity values were observed. Li and Irvine [83] and Carrasquero et al. [84] noted a decrease in alkalinity during the aerobic phase in a batch reactor, due to its consumption during the nitrification process. This confirms that during the treatment of the effluent with an HRT of 15 days, the nitrification phenomenon did not occur, as the typical decrease in alkalinity was not observed.

After evaluating the different HRTs and SRTs in the SBRs, and based on the efficiencies obtained for organic matter, nutrients, color, and turbidity, the best operational conditions were selected as an SRT of 8 h, an HRT of 25 days, and an aerobic operational sequence. Using an HRT of 25 days implies lower biomass generation through sludge purge compared to an HRT of 15 days; it also allows for the occurrence of the nitrification process in the SBR, which enhances the removal of ammoniacal nitrogen and TKN. An SRT of 8 h was selected because it produces an effluent that complies with Venezuelan regulations for both COD and BOD while processing a larger volume of wastewater in less time compared to a 10-h SRT, which would result in energy and oxygen supply savings in the reactor.

With these operational conditions, the next phase of the research on effluent from vegetable processing was conducted, focusing on evaluating whether aerated filling allows for a reduction in the SRT and increases the removal rates of organic matter and nutrients.

Treated water can be used for irrigation, particularly after undergoing a polishing treatment to reduce color and turbidity. This final treatment step ensures that the water meets the quality standards required for agricultural use, improving its clarity and eliminating suspended particles that could clog irrigation systems or affect soil permeability. The reductions in color and turbidity also minimize any negative perception regarding the water's quality, making it more acceptable for reuse. Utilizing treated water for irrigation promotes sustainable water resource management, especially in regions facing water scarcity, by reducing the demand for freshwater. Additionally, it supports agricultural

productivity while contributing to the circular economy, transforming wastewater into a valuable resource and fostering environmental conservation.

3.6. Limitations

This study was conducted using lab-scale sequencing batch reactors (SBRs), which provide valuable insights but may not fully represent industrial-scale operations. Scaling up could present challenges such as variations in flow rates, increased operational complexity, and higher costs associated with maintaining optimal conditions in larger systems.

The experiments were performed under controlled conditions (e.g., temperature, pH, and aeration). In real-world applications, fluctuations in environmental conditions, such as temperature variations or changing pH levels, could impact the system's performance, particularly for biological treatment processes that rely on stable microbial communities.

While this study focused on the removal of organic matter, it is important to note that some of the organic compounds were found to be non-biodegradable (refractory). Further research could explore the removal of these refractory compounds through advanced treatment technologies, as the current setup may not be sufficient to eliminate persistent pollutants.

This study primarily examined COD and BOD removal, which are important indicators of water quality, but do not provide a complete picture. Other emerging contaminants, such as microplastics, pharmaceuticals, or heavy metals, which may also be present in food processing wastewater, were not addressed.

This study was based on Venezuelan regulations, and the results may not be fully applicable in regions with stricter or different environmental standards, particularly regarding nutrient discharge limits or total suspended solids.

4. Conclusions

The SBR demonstrated high efficiency in removing organic matter, with COD removal rates between 75.9% and 84.8% and BOD removal exceeding 90% when operating under an 8-h operational cycle time (OCT) and a 25-day sludge retention time (SRT). These results confirm the reactor's capacity to produce treated effluents that comply with Venezuelan discharge regulations, emphasizing the practical feasibility of implementing this system in similar industrial settings.

Organic matter fractionation revealed that 80.6% of the COD was biodegradable, with 54.3% classified as readily biodegradable. This detailed analysis underscores the importance of understanding the organic load composition to optimize treatment strategies, enabling the efficient design of biological systems for food industry wastewater.

This study demonstrated that nitrogen removal occurred primarily via assimilation under aerobic conditions, especially at shorter SRTs, while nitrification processes became more dominant at longer SRTs (25 days).

The addition of nitrogen and phosphorus as macronutrients did not significantly improve organic matter removal, indicating that the microbial biomass present could maintain treatment efficiency under nutrient-limited conditions. This result provides practical insights into reducing operational costs by minimizing external nutrient supplementation.

The findings confirm that SBRs are a robust and flexible treatment option for vegetable processing wastewater that can adapt to fluctuating loads and achieving stable operational performance. From a practical perspective, this research provides a basis for industries to implement cost-effective, environmentally sustainable wastewater treatment systems that align with regulatory compliance and water resource conservation goals.

This study contributes to the understanding of biological processes in SBRs, particularly the kinetics of organic matter degradation and the mechanisms of nutrient removal.

The results serve as a foundation for further research into optimizing operational parameters, such as cycle times and sludge age, to improve treatment performance and energy efficiency in food processing industries.

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