



Influence of poultry and swine blood shocks on the performance of microalgal heterotrophic bioreactor

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ABSTRACT

Slaughterhouse blood is an inevitable part of the meat production chain and represents a problematic by-product due to the high volumes generated, and it is very high pollutant load. When discarded directly into the wastewater treatment facilities, they may cause abrupt variations in effluent concentration compromising the performance of biological wastewater treatment. In this sense, the objective of this work was to assess the performance of heterotrophic microalgal bioreactor in the poultry and swine slaughterhouse wastewater treatment when submitted to organic shock loads. The experiments were performed in a bubble column bioreactor, operating at 25°C, pH of 7.5, 100 mg/L of *Phormidium autumnale*, aeration of 1 VVM (volume of air per volume of culture per minute), and the absence of light. The organic shock loads were performed with 0.5%, 1%, 3%, 5%, 7%, and 10% of a mixture of poultry and swine blood (50% v/v) in a residence time of 48 h. The kinetic data of cell growth and substrate consumption have been collected and analyzed. The results indicate a pronounced variation in removal efficiency as a function of evaluated pollutant (chemical oxygen demand 95.57%–98.90%; total nitrogen 61.85%–88.23%; total phosphorus 77.82%–90.64%). The numerical indices of process performance, besides framing the wastewater from the point of view of international legislation, demonstrate that the bioreactor support organic shock loads typical to that of the poultry and swine slaughterhouse industry can generate.

Keywords: Agroindustrial wastewater; Slaughterhouse; Microalgae; Cyanobacteria; Organic loading rate; Organic shock load

1. Introduction

The poultry and swine slaughterhouse industries are an important world economic activity, and they generate a large volume of wastewater with a high pollutant load. It is estimated that this industrial process demands an average water volume of 10 m³ per ton of final product, leading to a high volume of wastewater requiring treatment [1]. Several types of disturbances can manifest in the case of industrial wastewater, even under normal operational conditions, given that the flow rate and concentration of organic matter vary with the industrial processes routine [2,3].

Blood produced in slaughterhouses represents a problematic by-product of the meat industry. The reuse of blood in by-products is considered the most important measure in reducing the biological oxygen demand (BOD) and chemical oxygen demand (COD) of slaughterhouse wastewater [4]. However, a portion of the blood is not captured and eventually leaves the processing floor along with water from the scalding or chill tanks or facility cleaning water and this extremely complex wastewater is eventually discharged into the environment [5,6].

A number of key operational parameters and environmental factors including organic shock load (OSL), solid retention time, pH, temperature, and toxicants can upset the process stability of a biological system, either temporarily or permanently. However, the likelihood of variations in

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the OSL is of prime importance in designing and operating wastewater treatment plants [7]. The magnitude, duration, and composition of the OSL predominantly determine the time necessary for the recovery of a microbiological community back to its stable conditions observed before the perturbation. Some biological systems show a certain degree of tolerance to perturbations, and it is important to evaluate and understand how the system can regain stability before the adverse effects become irreversible [8].

The activated sludge (AS), anaerobic digestion, and nitrification–denitrification processes are currently the most applicable technologies for the treatment of wastewaters [9,10]. However, they are biological processes that depend on mutual metabolic interactions among functional groups of microorganisms [9,11,12]. These microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. Failure to maintain the balance between these groups of microorganisms is the primary cause of reactors instabilities [7,11]. Conversely, the microalgal heterotrophic bioreactors are a potential technology to be applied in industrial wastewater treatment facilities due to their great efficiency in the removal of organic matter and nutrients as well as the possibility of the valorization of the wastes by nutrients cycling [13–15]. One characteristic of heterotrophic microalgal metabolism is the simultaneous conversion of the pollutants present in wastewater in a single step, thereby reducing the capital and operational costs [16].

Phormidium is a genus of filamentous, unbranched cyanobacteria, with filaments with a diameter of 3–4 μm and shows considerable potential for use as biocatalysts in environmental biotechnology processes because of their robustness and simple nutritional requirements [17,18].

The use of *Phormidium autumnale* in heterotrophic microalgal bioreactors for wastewater treatment could provide potential energy saving advantages of aeration of 3.52 W/m^3 compared with an AS process, representing an economy of USD 3.1 m^{-3} [19]. According to Santos et al. [16], the economic analysis of *P. autumnale* in the treatment of wastewater showed a cost of USD 2.7 m^{-3} . In addition, the potential production of bioproducts from microalgal sludge could contribute to development of multipurpose microalgal bioprocess concept [20].

In this regard, this study aimed to assess the OSLs on the performance of microalgal heterotrophic bioreactors in the treatment of poultry and swine slaughterhouse wastewater.

2. Materials and methods

2.1. Microorganisms and culture medium

A monoculture of *P. autumnale* was originally isolated from the Cuatro Ciénegas desert (26°59'N, 102°03'W-Mexico). Stock cultures were propagated and maintained in solidified agar–agar (20 g/L) containing synthetic BG11 medium [21]. The incubation conditions used were 25°C, a photon flux density of 15 $\mu\text{mol}/\text{m}^2/\text{s}$, and a photoperiod of 12:12 h (light:dark).

2.2. Wastewater

The poultry and swine slaughterhouse wastewater used in the experiments was obtained from an industry located in

Santa Catarina, Brazil (27°14'02"S, 52°01'40"W). It was collected from the discharge point of an equalization tank over a period of 1 year and analyzed for pH, COD, total nitrogen (N-TKN), total phosphorus (P- PO_4^{3-}), total solids (TS), suspended solids (SS), volatile solids (VS), and fixed solids (FS) following the Standard Methods for the Examination of Water and Wastewater [22]. The carbon/nitrogen (C/N) and nitrogen/phosphorus (N/P) ratios were calculated from the COD, N-TKN, and P- PO_4^{3-} , and adjusted when necessary with glucose. The average composition of the wastewater is shown in Table 1.

2.3. Obtaining kinetic parameters in bioreactor

Measurements were made in a bubble column bioreactor. The system was built of borosilicate glass and had an external diameter of 12.5 cm and a height of 16 cm, resulting in a height/diameter (h/D) ratio equal to 1.28 and a nominal working volume of 2.0 L. The dispersion system of the reactor consisted of a 2.5 cm diameter air diffuser located inside the bioreactor. The airflow was monitored by a flow meter (KI-Key Instruments®, Trevose, PA, USA) as shown in Fig. 1.

The experiments were performed in bioreactors, operating in a batch system, fed to 2.0 L of the poultry and swine slaughterhouse wastewater. The operational conditions were an initial cell concentration of 100 mg/L, constant aeration of 1.0 volume of air per volume of culture per minute (VVM), pH adjusted to 7.6, temperature 25°C, and the absence of light [16].

The OSLs of 0.5%, 1%, 3%, 5%, 7%, and 10% were performed with a mixture of poultry and swine blood (50% v/v) in a residence time of 48 h, corresponding to the end of the logarithmic growth phase. The blood composition is shown in Table 1.

2.4. Sampling and analytical methods

Samples were collected at regular intervals of 24 h and characterized for the COD, N-TKN, P- PO_4^{3-} , and cell biomass. The COD, N-TKN, and P- PO_4^{3-} were determined according to the methodology previously described in Section 2.2. Cell biomass was determined gravimetrically, filtering a known volume of culture through a 0.45 μm membrane filter

Table 1
Composition of poultry and swine slaughterhouse wastewater and blood mixture

Parameter	Wastewater	Blood
pH	5.90 \pm 0.05	7.4 \pm 0.01
COD (mg/L)	2,100.00 \pm 874.00	38,533.00 \pm 1,200.00
N-TKN (mg/L)	68.50 \pm 12.10	2,781.15 \pm 135.00
P- PO_4^{3-} (mg/L)	8.48 \pm 4.02	270.00 \pm 13.20
TS (mg/L)	3.80 \pm 2.70	40.44 \pm 4.57
SS (mg/L)	1.90 \pm 0.81	8.79 \pm 2.43
VS (mg/L)	2.90 \pm 1.42	37.01 \pm 1.00
FS (mg/L)	0.90 \pm 0.31	3.42 \pm 2.16
C/N	30.85 \pm 0.30	13.85 \pm 1.42
N/P	8.07 \pm 0.54	10.30 \pm 0.90

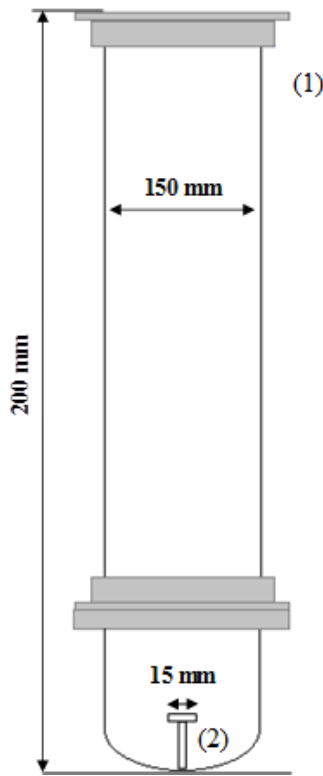


Fig. 1. Schematic diagram of the bioreactor: (1) bioreactor and (2) air diffuser.

(Millex FC®, Billerica-MA, USA), and drying at 60°C for 24 h. Tests were performed twice and in duplicate. Therefore, experimental data refer to the mean value of four repetitions.

External contamination has been monitored by the heterotrophic plate count method, according to Maroneze et al. [17].

3. Results and discussion

The separation of the carbon, nitrogen, and phosphorus removal stages results in cost increases of the technological control of conventional wastewater treatment systems. The use of microalgal heterotrophic bioreactor as an alternative allows the simultaneous conversion of pollutants present in wastewater in a single step, thereby reducing capital and operational costs [23].

In this sense, Fig. 2 shows the dynamics of cell biomass as well as the consumption of the organic carbon, nitrogen, and phosphorus in normal operating conditions in a slaughterhouse. The analysis of the results shows a maximum cell biomass of 1,720 mg/L and efficiencies of the removal of COD, N-TKN, and P-PO₄⁻³ of 94.35%, 79.57%, and 91.65%, respectively, which demonstrates the ability of a microalgal heterotrophic bioreactor to remove the three pollutants in a single step.

The absence of an adaptation phase is shown in parallel with a logarithmic phase of growth with duration of 48 h. After the logarithmic growth phase (48 h), the cell growth was stabilized; however, the organic matter and nutrients continued to reduce until the residence time of 72 h. These reductions in substrate consumption are related to

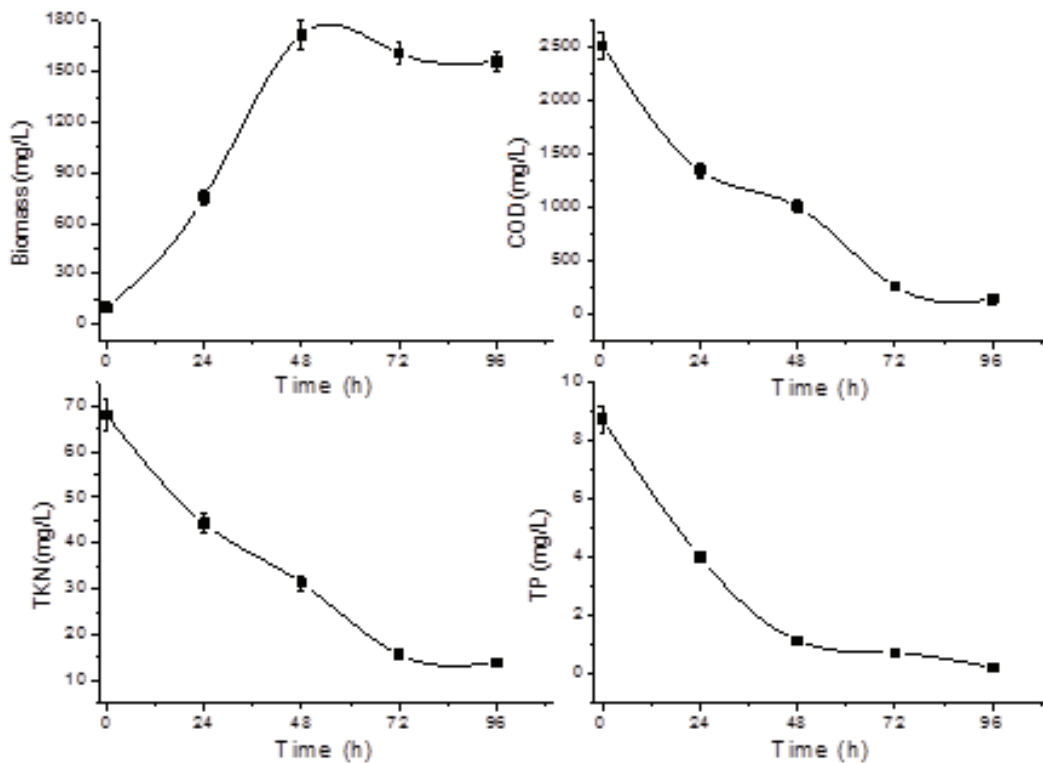


Fig. 2. Dynamics of cell biomass, organic carbon (COD), nitrogen (N-TKN), and phosphorus (P-PO₄⁻³) in the control experiment (without organic shock load).

maintaining metabolism of microorganism, which include the turnover of cell materials, osmotic work to maintain concentration gradients and cell motility, which corresponds to metabolic requirements inferred for a zero growth condition [24–26]. Thus, the cell growth stabilization occurs because organic pollutants are diverted from assimilation via biosynthesis to energy-requiring functions associated with nongrowth activities [27]. In addition, other mechanisms capable of eliminating nitrogen and phosphorus in intensively aerated microalgal systems are nonbiological, such as air stripping, ammonia volatilization, absorption, and sedimentation [28–30].

According to the Food and Agriculture Organization of the United Nations [31], approximately 1.32 billion poultry

and 37.9 million swine were processed in Brazil in 2016. Considering that, even while under normal operational conditions, the blood supply of the 0.075 L/poultry and 0.5 L/swine into the wastewater may eventually occur, and this would amount to an annual blood supply to the wastewater of the 117.9 million L. For an appropriate design of a treatment plant, it is necessary to provide a rational description of related processes in terms of microbial kinetics, emphasizing the required wastewater characterization for the assessment of biological treatability [32]. In this sense, Fig. 3 and Table 2 show the dynamics of cell growth and pollutants, besides the performance parameters of process submitted to OSLs.

The results shown point out that OSLs of 0.5%, 1%, and 3% had maximum cell biomass (X_{max}) and average biomass

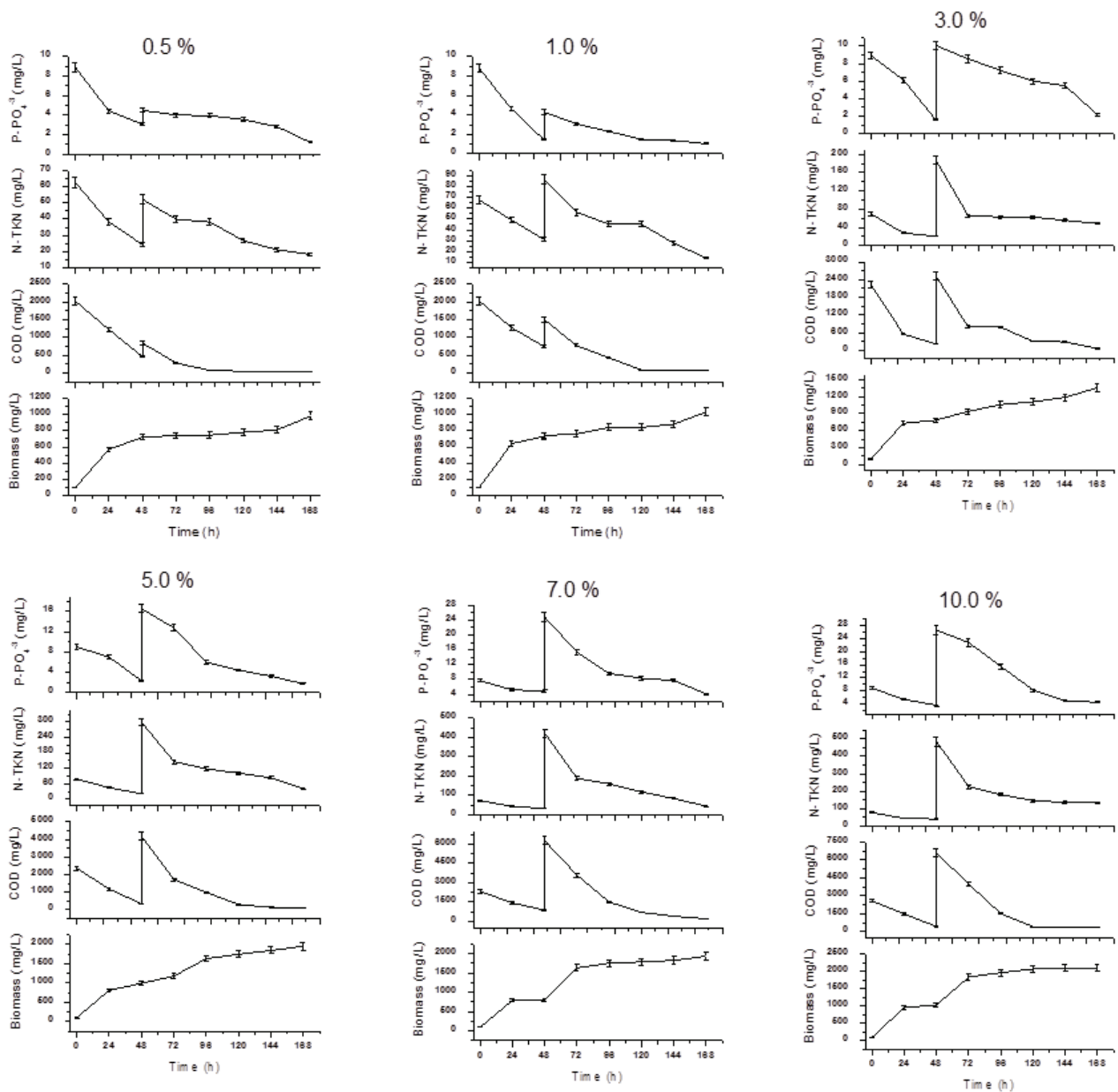


Fig. 3. Dynamics of cell biomass, organic carbon (COD), nitrogen (N-TKN), and phosphorus ($P-PO_4^{3-}$) with organic shock loads of 0.5%, 1%, 3%, 5%, 7%, and 10% performed at a residence time of 48 h.

Table 2

Cell growth parameters, removal efficiency, and final concentration of organic carbon (COD), nitrogen (N-TKN), and phosphorus (P-PO₄⁻³) in control experiment (without organic shock load) and different organic shock loads

Experimental condition	Cell growth		Removal efficiency (%)			Final concentration (mg/L)		
	X _{max} (mg/L)	P _x (mg/L)	COD	N-TKN	P-PO ₄ ⁻³	COD	N-TKN	P-PO ₄ ⁻³
Control	1,720 ± 144	33.75 ± 1.57	94.36 ± 0.15	79.57 ± 0.88	97.48 ± 0.62	142.33 ± 0.87	13.90 ± 0.16	0.22 ± 0.54
OSL 0.5%	980 ± 78	5.23 ± 0.45	98.90 ± 0.00	61.85 ± 1.55	80.59 ± 1.02	21.30 ± 0.56	18.24 ± 0.86	1.18 ± 0.66
OSL 1.0%	1,030 ± 106	5.53 ± 0.73	96.05 ± 0.10	82.23 ± 1.00	88.5 ± 1.24	78.00 ± 0.80	13.90 ± 1.20	1.05 ± 1.00
OSL 3.0%	1,360 ± 45	7.50 ± 0.19	98.40 ± 0.22	70.83 ± 0.90	84.24 ± 1.10	68.74 ± 1.00	48.67 ± 1.00	2.14 ± 0.90
OSL 5.0%	1,950 ± 185	11.01 ± 1.68	98.82 ± 0.34	85.88 ± 0.55	90.64 ± 1.65	68.66 ± 0.50	41.40 ± 0.80	1.79 ± 0.45
OSL 7.0%	1,940 ± 123	10.95 ± 1.61	96.92 ± 0.60	88.23 ± 1.85	77.82 ± 1.00	205.00 ± 1.05	45.19 ± 1.20	4.13 ± 1.00
OSL 10%	2,090 ± 154	11.84 ± 1.64	95.57 ± 0.55	62.22 ± 1.20	80.12 ± 1.40	352.00 ± 1.25	132.00 ± 0.75	4.64 ± 1.10

productivity (P_x) of 980 and 5.23 mg/L h, 1,030 mg/L and 5.53 mg/L h, and 1,360 mg/L and 7.5 mg/L h, respectively. These values are lower than those presented by control experiment, where the X_{max} and P_x were 1,790 mg/L and 33.75 mg/L h, respectively (Fig. 1). However, OSLs of 5%, 7%, and 10% showed an increase in X_{max} compared with control, presenting values of 1,940, 1,950, and 2,090 mg/L, respectively. This is related to a higher concentration of organic matter, which can enable microalgae to maintain cell growth [33]. It is also noted a pronounced variability in the removal efficiencies (REs) is observed as a function of the evaluated pollutant, obtaining REs of organic carbon, nitrogen, and phosphorus ranging from 95.57% to 98.9%, 61.85% to 88.23%, and 77.82% to 90.64%, respectively. Values of REs for carbon are higher to those found in conventional processes such as anaerobic digestion, with REs of 85%–92.6% [34], and AS, with REs of 93.5%–97.2% [35] for slaughterhouse wastewater treatment. These results demonstrate that the microalgal heterotrophic bioreactor, besides supporting OSLs, can increase the production of microalgal biomass, potentializing its reuse [20].

Environmental legislation is necessary to mitigate the environmental impact of the slaughterhouses, and the treatment methods are used as the main regulatory requirement [36]. In this sense, still according to Table 2, the results for the final concentrations of organic carbon, nitrogen, and phosphorus varied from 21.30 to 352 mg/L for COD, from 12.9 to 132 mg/L for N-TKN, and from 1.05 to 4.64 mg/L for P-PO₄⁻³. From the point of view of compliance with current legislation, only the OSLs of 0.5%, 1%, 3%, and 5% comply with the emission standards for organic carbon [37,38]. From the analysis of the results of the final concentrations of nitrogen and phosphorus, it is possible to verify that the proposed system is not in conformity with the final concentrations required by the current legislation. However, from the point of view of RE, the minimum percentage of reduction of the pollutant load for all urban wastewater treatment plants is 75% for N-TKN and P-PO₄⁻³. In this sense, the OSLs of 1%, 5%, and 7% comply with the established value for nitrogen and all OSLs comply with the established value for phosphorus [37,38].

Based on these results, the proposed process showed potential application, since if all blood in a typical slaughter line, where 450,000 poultry and 2,000 swine are slaughtered per day, was discharged to a wastewater treatment plant with a capacity of 16,000 m³/d, the concentration of blood ratio would be around 1%. In this concentration of OSL,

the process reached the concentration of COD, N-TKN, and P-PO₄⁻³ below the minimum allowed according to the legislation [37,38].

The conventional biological processes used for the treatment of wastewater can be adversely affected by variations in the flow and concentration of organic load, since they are unable to process imbalances [3,9,39]. In addition, these processes convert only organic material, and part of the nitrogen and phosphorus material, contributing to the partial removal of nutrients from wastewater, requiring subsequent operations to remove these pollutants [40].

Comparatively, Chelliapan et al. [41], when evaluating the influence of OSLs on the microbial community performance of an upflow anaerobic stage reactor, for the treatment of pharmaceutical wastewater, found 70% COD RE in an OSL of 430 mg/L COD, performed in four steps, totaling 1,720 mg/L of COD. However, increasing the OSL to 1,860 mg/L COD, performed in four steps, totaling 7,440 mg/L of COD reduced the COD RE of 45%. Alves et al. [42] evaluated the effect of the OSL on the performance of an AS and moving bed biofilm reactor (MBBR) system. The AS-MBBR system withstands OSLs of up to 2,720 mg/L COD, performed in three steps, totaling 8,160 mg/L COD, above which its performance was disturbed. Nitrifiers showed higher sensitivity to organic matter than heterotrophs, which are already impaired at 1,800 mg/L COD.

The results showed that the wastewater treatment in a microalgal heterotrophic bioreactor is able to withstand OSLs of 385.3–6,165 mg/L COD, performed in only one step, obtaining efficiencies of removal of organic carbon between 95.57% and 98.9%, besides efficiently removing nitrogen and phosphorus in parallel.

The aseptic procedures adopted have been suitable for preventing microbial contamination of the cultures (data not shown), since null results have been observed through the heterotrophic plate count method. Although the use of single specie of microorganism has the advantages early reported, the control and maintenance of the external contamination by other heterotrophic microorganisms is one the main limitations of this technology. The maintenance of a monoculture in full scale is prohibitively expensive and technically difficult to operate. In this sense, improving microalgae culture stability is a challenge to be surmounted before the industrial application of microalgal heterotrophic bioreactors in wastewater treatment facilities [43].

4. Conclusion

The results suggest that the microalgal heterotrophic bioreactor is a potential technology in wastewater treatment facilities, since it was able to withstand the OSLs that a poultry and swine slaughterhouse can generate. In addition, this technologic route has advantages over conventional biological treatments, since they remove organic matter and nutrients in a single step.

Based on these results, the proposed process showed potential application, since that OSLs of 1% (typical imbalance found in poultry and swine wastewater treatment plants) were tolerated by the heterotrophic microalgal bioreactor (REs of COD 96.05%, N-TKN 82.23%, and P-PO₄⁻³ 88.5%).

Symbols

OSL	—	Organic shock load
BOD	—	Biological oxygen demand
VVM	—	Volume of air per volume of wastewater per minute
COD	—	Chemical oxygen demand (mg/L)
N-TKN	—	Total nitrogen (mg/L)
P-PO ₄ ⁻³	—	Total phosphorus (mg/L)
TS	—	Total solids (mg/L)
SS	—	Suspended solids (mg/L)
VS	—	Volatile solids (mg/L)
FS	—	Fixed solids (mg/L)
C/N	—	Carbon/nitrogen ratio
N/P	—	Nitrogen/phosphorous ratio
X _{max}	—	Maximum cell biomass
P _x	—	Biomass productivity (mg/L)
RE	—	Removal efficiency (%)

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