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Craft Brewery Wastewater Treatment: a Fixed-Bed Single-Batch Reactor with Intermittent Aeration to Remove COD and TN

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HIGHLIGHTS

- A fixed-bed single-batch reactor with intermittent aeration was used to remove COD and TN from brewery wastewater.
- The surface response from CCD showed that, when using the same HRT, the higher the aeration time the higher the efficiency.
- Good quality effluent was obtained with 20 h HRT and 3 h aeration time, in a 4 h cycle.
- Polyurethane foam (Mini Biobob©) is an adequate biofilm media support to promote the SND process.
- The correction of influent alkalinity promoted better COD and TN removal efficiencies than with no such correction.

Abstract: This study evaluated an intermittently aerated, fixed-bed, single-batch reactor, with mini BioBob© as biofilm media support, as an alternative treatment of craft brewery wastewater. In order to remove chemical oxygen demand (COD) and total nitrogen (TN), seven conditions were performed in a central composite experimental design (CCD) with different aeration times (1, 2 and 3 h in a 4 h cycle) and hydraulic retention times (HRT) (12, 16 and 20 h). The results showed that the removal of COD and TN were positively affected by increased aeration time and HRT. The condition that presented the best quality effluent was Condition No. 1 (20 h HRT and 3 h aeration), with 209 ± 28 mg COD L^{-1}; 3.00 ± 0.15 mg TKN L^{-1}; and 0.67 ± 0.11 mg NO_3-N L^{-1}. Kinetic assays showed that the highest values for the substrate removal rate constant, k_{COD} = 0.1774 h^{-1} were obtained with the longest aeration time (3 h). The most probable number (MPN) test showed a higher concentration of denitrifying bacteria (heterotrophic), 3.3 x 10^6, than for AOB and NOB bacteria (autotrophic), which were 4.9 x 10^3 and 2.7 x 10^3, respectively. Moreover, it was possible to verify that correcting the influent alkalinity with 7.14 mg $CaCO_3$ for each 1 mg of TKN resulted in better process efficiency. It was concluded that COD and TN can be removed from craft brewery wastewater using an intermittently aerated, fixed-bed, single-batch reactor with mini Biobob© as biofilm media support.

Keywords: simultaneous nitrification and denitrification; polyurethane foam; MPN method; mini Biobob®.

INTRODUCTION

Beer is the most consumed alcoholic beverage in the world [1]. In 2014, global beer production reached 119 million kiloliters. According to the Food and Agriculture Organization of the United Nations, Brazil is one of the largest beer producers, contributing about 14 million kiloliters [2].

Wastewater from breweries contains high concentrations of organic matter, which is composed of protein, carbohydrates, ethanol and suspended solids. Approximately 3 to 10 liters of wastewater is generated per liter of beer that is produced [3-5]. This wastewater is generated in different process stages (filtration, fermentation, washing and sanitization), resulting in potential environmental pollution if appropriate treatments are not performed [6,7]. Table 1 presents some physical-chemical characteristics of brewery wastewater.

Table 1. Characterization of brewery wastewater [3, 8-10].

Parameter	Value
pH	3-12
Temperature (°C)	18-40
COD (mg L^{-1})	2,000-10,000
BOD (mg L^{-1})	1,200-3,600
TN (mg L^{-1})	25-80
TS (mg L^{-1})	5,100-8750
TP (mg L^{-1})	10-50

Craft brewery effluent is characterized by high COD and TN content [8]. This is because bottle washing, a step that does not occur in craft breweries, results in large volumes of wastewater, but with lower concentrations of organic matter [3]. Many craft breweries are also located in areas with no access to a sewage collection system and, consequently, a compact wastewater treatment system could provide operational and financial advantages [9,10].

Studies using biological treatment, with aerobic and anaerobic processes to treat brewery wastewater, have been performed and have demonstrated that they are adequate for COD reduction [11-14]. Typical waste water treatment plants (WWTP) for brewery wastewater use biological methods. Anaerobic digestion, with methane production, followed by an active sludge system, is the standard and most recommended process for breweries [3,14]. In addition, in conventional effluent treatment processes that involve the removal of nitrogen and organic matter, the nitrification and denitrification phases take place in separate environments, which makes system implementation and monitoring more expensive [3] and, consequently, more difficult for small breweries to use. Other studies have focused on fungal cultivation to remove organic carbon, phosphorus and nitrogen from brewery wastewater [5].

Microbreweries produce in a discontinuous way; therefore, a discontinuous system to treat wastewater could be more advantageous than a continuous system. Batch reactors have the advantage of producing good effluent quality due to the following factors: the process can only be finished when standard emission has been reached; there are no primary or second settler; and they provide simple and stable operation processes. Furthermore, they can also be used by industries that generate wastewater in discontinuous mode [15] such as small craft breweries.

It is hoped that compact WWTP, with a one-flow diagram based on N-removal, can be incorporated within mainstream technologies for wastewater treatment in the future [16]. Studies of single reactors that provide aerobic and anaerobic zones in order to simultaneously remove TN and COD have generated interest, and a number of studies using different types of wastewater have already been performed [16-18].

Several studies have used polyurethane (PU) foam as support for biofilm development, which provides good fixation of the microorganisms and improved COD and NT removal in a single-step reactor [17-22]. PU foam promotes substrate and gradient of electron donor, which allows the occurrence of simultaneous nitrification and denitrification (SND) in a single media [17-19].

Araujo Júnior and coauthors [12] evaluated the removal of COD from brewery wastewater in a continuous reactor using two different biomass support materials (polyurethane and polypropylene); they concluded that the best efficiency was obtained with polyurethane foam support.

To date, there has been no study concerning the use of single-batch reactors with PU foam to remove TN and COD from brewery wastewater. In the present study, a fixed-bed, single-batch reactor with PU foam was operated with different aeration times and HRT in order to (i) evaluate its performance regarding COD and TN removal; and (ii) to assess the COD and TN consumption rates as a function of aeration time.

MATERIALS AND METHODS

Fixed-bed, single-batch reactor

The reactor was an acrylic compartment of 60.0 cm height and 14.5 cm internal diameter, with total volume of 10.0 L and working volume of 6.0 L. The reactor was filled with 947 units of Mini Biobob© media (Bioproj Tecnologia Ambiental) that occupied 8.0 L liters (Figure 1). To ensure mixing in the reactor, a recirculation system was performed using a Concept Plus Prominent solenoid pump with a flow rate of 2.1 L h^{-1}. The recirculated effluent left the top and entered at the bottom part of the reactor. The internal temperature was maintained at 30 °C. Aeration was performed by three aquarium air pumps (Boyu SC-3500, 2.5 W) with hoses attached to porous stones to improve air diffusion in the liquid.

The Mini Biobob© carriers had a cylindrical design, with 1.5 cm diameter and 2.0 cm length, an external frame made of high-density polyethylene (HDPE), and an inner part of polyurethane foam (PU) with 50% porosity and 28 kg m^{-3} density.

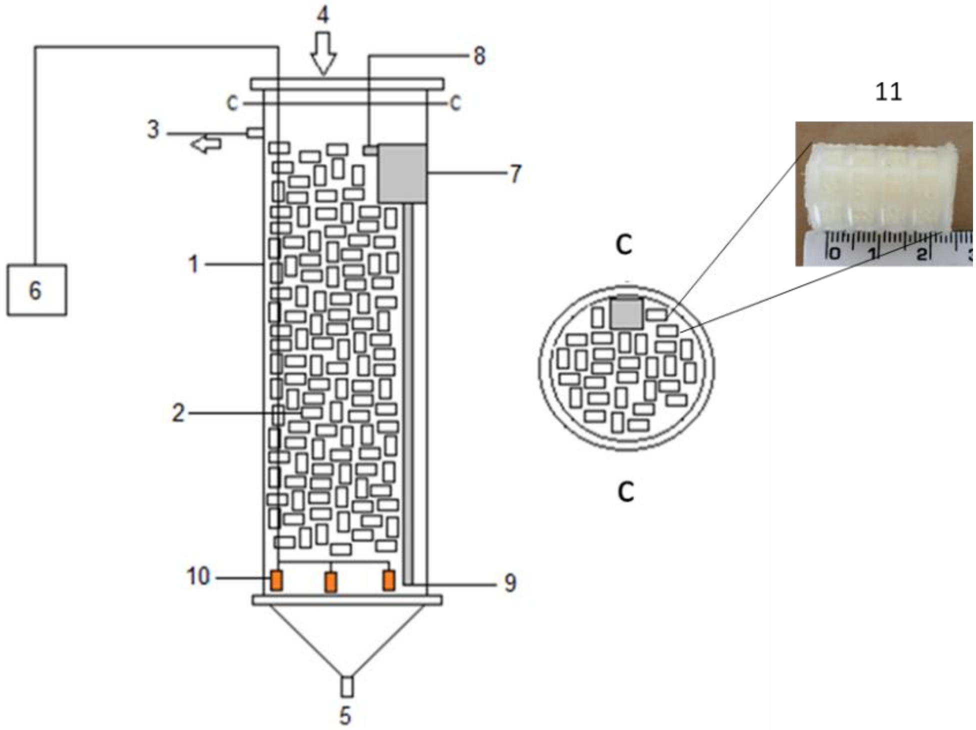

Figure 1. Schematic representation of the single fixed-bed reactor with recirculation and intermittent aeration: (1) reactor, (2) Mini Biobob© media, (3) sample collection, (4) influent feed, (5) effluent output, (6) aerator, (7) recirculation pump, (8) recirculation pump output, (9) recirculation pump inlet, (10) air diffusers, (11) Mini Biobob© carriers.

Brewery wastewater

The wastewater was collected from a small craft brewery that produces 20 types of beer, with no continuous or daily production. The brewing process involves several steps and the effluent is generated at different points in the process (Figure 2).

To avoid variations in the concentration of COD and TKN in the influent, only one lot of wastewater was collected at the mashing and boiling stages, before being diluted with washing and sanitation water. At this point of collection, the wastewater had a COD concentration of approximately 150,000 mg L^{-1}. This brewery generates about 30 L of this concentrated wastewater and 1,900 L of washing and sanitation wastewater for each batch, which are mixed before being discharged. Thus, before this experiment, the mashing and boiling wastewater was diluted with tap water at a ratio of 1:60 (v:v) to have a similar COD concentration to the real one, i.e. between 2,000 and 3,000 mg L^{-1}. The wastewater was collected in one morning and quickly taken to the laboratory, where it was stored at 8 °C.

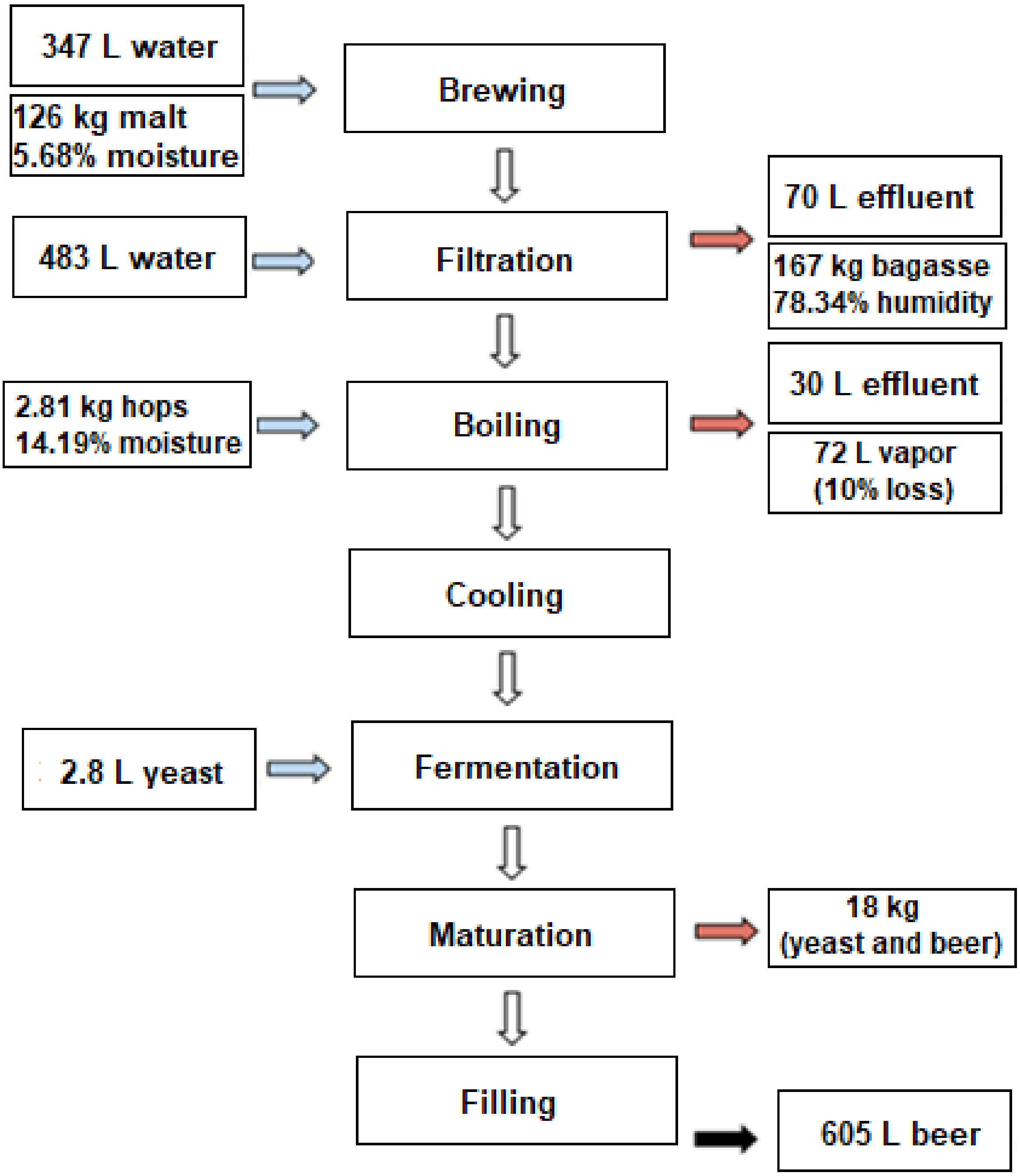

Figure 2. Flowchart and mass balance of the beer brewing process.

Inoculum

The inoculum used in this study was collected from an activated sludge reactor at the Heineken Brewery located in the city of Ponta Grossa, Paraná, Brazil. This inoculum was chosen due to the presence of nitrifying bacteria and also because it was already adapted for brewery wastewater. The immobilization of the inoculum was performed according to the protocol described by Zaiat and coauthors [23].

Reactor operation

The reactor operation was divided into three phases with a distinct duration for each one as follows: a) fill (0.2h); b) operation (HRT of 20, 16 or 12 h); and c) draw (0.1 h). During the filling and drawing phases the aeration and recirculation were switched off.

Experimental design and statistical analysis

To evaluate the effect of the factors (HRT and aeration) on the response variables (COD and TN removal), a central composite design (CCD) with a 2^k factorial design was used, where k was the number of factors, and 2 was the number of factor levels. The CCD consisted of seven tests, four of which were at levels +1 and -1, and three replicates at the central point. The conditions used to evaluate the TN and COD removal, as well as the nitrification and denitrification efficiency, are shown in Table 2.

The data obtained from the experimental design were analyzed using Statistica© 7 software. The data were first checked for normality by the Shapiro–Wilk test, and ANOVA tests were then performed. Normality tests are used to verify if the probability distribution associated with a data set could be approximated by the normal distribution [24].

Table 2. Independent variables (factors), HRT, and aeration times (coded and real values).

Condition	Coded values		Real values	
	HRT	Aeration	HRT (h)	Aeration On/Off (h)
1	1	1	20	3/1
2	1	-1	20	1/3
3	-1	1	12	3/1
4	-1	-1	12	1/3
5	0	0	16	2/2
6	0	0	16	2/2
7	0	0	16	2/2

Each condition needed five days for the bacteria to acclimatize. It was only after this period, during steady state condition, that the data for the evaluation of reactor performance was considered. The biomass was considered to be adapted when it was possible to note the presence of nitrate, indicating that nitrifying bacteria were acting.

Analytical methods

To evaluate the bioreactor performance, the chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and pH were analyzed according to APHA [25]. Alkalinity was measured according to DiLallo [26].

Alkalinity correction

Autotrophic nitrification and heterotrophic denitrification rely on alkalinity. The nitrification process consumes 7.14 mg of $CaCO_3$ for each 1 mg of TKN. During denitrification, 3.57 mg of $CaCO_3$ return to the system for each 1 mg of reduced NO_3^--N. Without alkalinity, acids formed during this process would lead to a fall in the pH, which would inhibit the bacteria activity. Thus, before feeding the reactor, the influent alkalinity was corrected to the appropriate $CaCO_3$ concentration.

The need to add an alkalinizing agent makes the process more expensive. Consequently, in order to investigate the influence of alkalinity on the performance of the system, the best condition was repeated, but with no alkalinity correction.

Most probably number (MPN)

After all the studied conditions were concluded, the best condition was performed again to quantify the presence of nitrifying and denitrifying microorganisms using the most probable number (MPN) method.

For the nitrifying microorganisms the methodology used was based on Schmidt and coauthors [27], and for the denitrifying microorganisms the methodology described by Tiedje and coauthors [28] was used. The estimation of the MPN was obtained from the combination of positive tube results with the standard probability table [25].

Kinetic assays

In order to assess the COD and TN consumption rates as a function of aeration time, three kinetic tests were performed. Each test lasted 4 h with different aeration times of 3 h, 2 h and 1 h. The kinetic assays were performed in the reactor with the same influent that was utilized in the seven studied conditions. The experimental data obtained were analyzed graphically (reaction rate vs substrate concentration), an equation was fitted, and the order (n) of the reaction was determined. After determining the order of the reaction the integral method was used to determine the reaction rate constant (k).

Considering a reaction, A → product(s) performed in a constant volume batch reactor, the molar balance in the reactor results was as follows:

$$\frac{dCa}{dt} = -r_a \tag{1}$$

Integrating the equation, and knowing that for a zero-order, first-order and second-order reaction the value of r_a is k, k.Ca and k.Ca^2 respectively, for initial time and concentration (time = 0), we concluded:

- Zero-order (mg $L^{-1}h^{-1}$):

$$Ca = Cao - k.t \tag{2}$$

- First-order (h^{-1}):

$$\ln\left(\frac{Ca}{Cao}\right) = -k.t \tag{3}$$

- Second-order ($mg^{-1}L^{-1}h^{-1}$):

$$\frac{1}{Ca} - \frac{1}{Cao} = k.t \tag{4}$$

In the above equations, Cao and Ca were the respective substrate concentrations (mg L^{-1}) in the influent and effluent.

By plotting the graph for all the equations, the reaction rate constant (k) was determined through the angular coefficient. The reaction rate constant is expressed by k, and the time by the variable t (h).

RESULTS

COD and TN removal efficiencies

The average influent COD concentration was 2,374 mg L^{-1}. The effluent COD concentration ranged from 209 ± 28 to 1,129 ± 3 mg L^{-1} and the removal efficiency varied from 48% to 92%. Table 3 and Figure 3a show that an increase in aeration time and HRT positively contributed to COD removal. Table 3 shows that, when using the same HRT, the best COD reduction efficiency occurred in the conditions with the longest aeration time, i.e., 3 h. In Condition Nos. 1 and 2, with 20 h HRT, COD removal efficiencies of 92% and 71% were obtained for 3 h and 1 h of aeration, respectively. The same happened in Condition Nos. 3 and 4; with 12 h TDH there was COD removal efficiency of 71% and 48% with respective aeration of 3 h and 1 h.

The Pareto charts (Figure 4a), show that in terms of COD removal the factors of HRT and aeration time had a positive and significant effect.

Di Biase and coauthors [14], studied the performance of an anaerobic, moving-bed biofilm reactor (AMBBR) treating brewery wastewater. They obtained the highest performance of 92% removal of sCOD (soluble COD) with an OLR of 5.4 kg COD m^{-3} d^{-1}. When an OLR of 23.6 was applied the percentage of removal decreased to 65% sCOD. Compared with a study by di Biasi and coauthors [14], this reactor showed better results. This can be attributed to the aeration, which promotes better levels of COD removal than the anaerobic process in isolation.

Table 3. Organic load, influent, effluent and COD removal efficiency.

Condition	OLR (kg DQO $m^{-3}d^{-1}$)	Influent COD/N	Influent COD (mg L^{-1})	Effluent COD (mg L^{-1})	OLR_{rem} (kg DQO $m^{-3}d^{-1}$)	Removal efficiency (%)
1	19.3	107	2,682 ± 30	209 ± 28	17.8	92
2	18.7	111	2,596 ± 79	729 ± 67	13.4	71
3	26.1	108	2,175 ± 60	615 ± 7	18.7	71
4	26.3	109	2,196 ± 17	1,129 ± 3	26.3	48
5	18.6	98	2,066 ± 18	579 ± 3	13.4	71
6	19.8	110	2,207 ± 32	571 ± 32	14.7	74
7	19.7	104	2,196 ± 67	564 ± 6	14.7	74

Bakare and coauthors [13] studied the treatment of brewery wastewater with two types of operational aeration: intermittent aeration with an OD concentration of 3.0 mg L^{-1}; and continuous low aeration with an OD concentration of 1.5 mg L^{-1}. They obtained COD removal efficiency of 90% with constant aeration, and 78% for the system with intermittent aeration. They concluded that the better performance obtained under continuous low aeration was due to the constant availability of oxygen, which improved microbial activity. It can be inferred that the same situation occurred in the present study. Furthermore, in the presence of high levels of organic matter and high concentrations of OD, aerobic heterotrophic bacteria can develop quickly, consuming high levels of COD.

Ozturk and coauthors [29] compared a sequencing batch reactor (SBR) and a sequencing batch biofilm reactor (SBBR) for the treatment of dairy wastewater. The SBBR was filled with Kaldnes K1 biocarrier. The authors concluded that adding biocarrier to the reactor promoted better COD removal. They observed COD removal efficiency of 63.5% for the SBR and 81.8% for the SBBR.

Araujo and coauthors [12] evaluated two anaerobic fixed-bed reactors for the treatment of brewery wastewater. Two different types of biomass support were tested, polypropylene (PP) and PU. The authors obtained the best global efficiency with PU as support. For an ORL with 14 kg COD m^{-3} d^{-1} (HRT of 8 h) and 20.3 kg COD m^{-3} d^{-1} (HRT of 12 h) the PU reactor reached average COD removal efficiencies of 81% and 71%, respectively. The aforementioned authors concluded that the high superficial area of the PU foam increased the fixed biomass concentration, contributing to the increase in reactor performance.

Khouni and coauthors [30] investigated the performance of an aerobic membrane bioreactor (MBR), with C/N varying from 10 to 30, and obtained COD removal rates ranging from 76% to 94%. According to these authors, no significant effect of the C/N ratio on COD removal was observed. The same was observed by Hao and Liao [31], who also concluded that aeration was more important than the COD/N ratio for COD removal.

In the present study, the only condition where the effluent met the standards required for COD (225 mg L^{-1}) was Condition No. 1, with 209 ± 28 mg L^{-1}. However, if a brewery in Brazil has permission to discharge effluent to a local treatment plant, pre-treatment of the wastewater can help to prevent COD overload in the WWTP, and the brewery does not need to reach the required standard required.

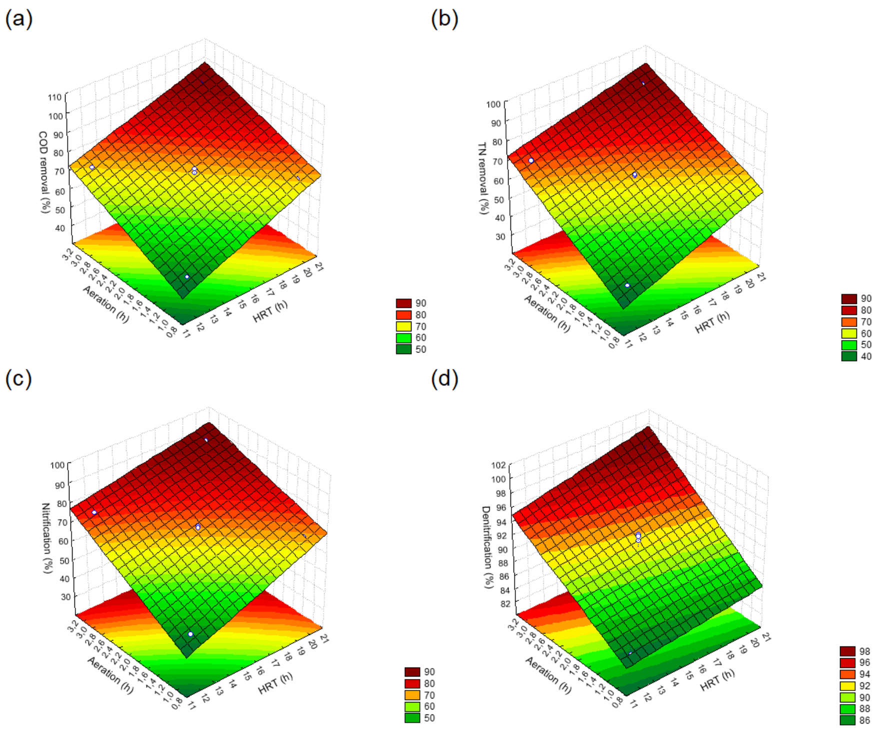

Figure 3. Response surface plots to show the effects of the factors of aeration and HRT on (a) COD removal, (b) TN removal, (c) nitrification efficiency and (d) denitrification efficiency.

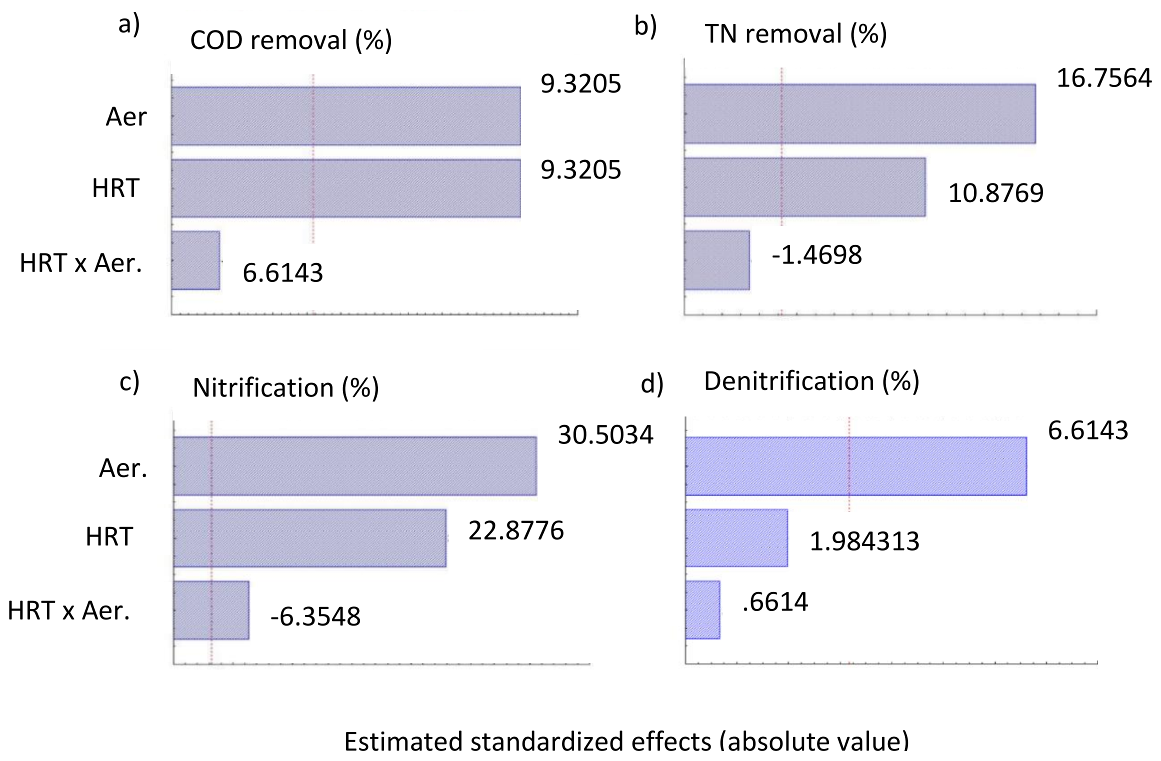

Figure 4. Pareto charts: a) COD removal, b) TN removal, c) nitrification and d) denitrification efficiency (p=0.05).

This study showed that TN removal, nitrification and denitrification efficiency (Figs. 4b, 4c and 4d, respectively were affected to a greater extent by aeration than by HRT. Comparing Condition Nos. 1 and 2, both of which used 20 h HRT (Table 4), it is possible to observe that Condition No 1 was more efficient, with 3 h of aeration and 85%, 88% and 97% of TN, nitrification and denitrification efficiency, respectively.

Moura and coauthors [19] studied a structured-bed reactor subjected to recirculation and intermittent aeration (SBRRIA) treating sewage. They also observed that nitrification had higher efficiency with a higher aeration time; however, the denitrification efficiency remained similar during all their experiments. The authors concluded that nitrification was affected by the OD concentration, highlighting the fact that nitrification was the limiting step for SND.

Although denitrification occurs in an environment without dissolved oxygen, it was possible to observe in the present study that denitrification occurred when nitrate and nitrite were present, in other words, the limiting step in relation to SND was nitrification. Leyva-Díaz and coauthors [32] suggest that nitrification is the limiting step in wastewater treatment systems because the community structure of denitrifying bacteria is much more versatile than the community structure of nitrifying bacteria.

Despite the high COD/NT ratio in the present study, SND occurred in this reactor because the Biobob© enabled the occurrence of aerobic and anoxic zones at different depths in the foam cylinders. Nitrification occurs where there is the presence of oxygen, in the outermost regions of the support medium, and aerobic nitrifying bacteria are present. The denitrifying facultative heterotrophic bacteria that consume COD, and reduce nitrite and nitrate to nitrogen gas, occur in deeper regions where oxygen cannot diffuse [21].

Ozturk and coauthors [29], studied dairy effluent with a COD/N ratio equal to 72; they observed the removal of ammonium by nitrification, with nitrate formation in an SBBR and an SBR aerobic reactor. Babatsouli and coauthors [33] evaluated a MBR treating industrial wastewater; they found a COD/N ratio of 80 and observed no accumulation of nitrate in the system, confirming the occurrence of SND.

Table 4. Mean values of TKN, NH_4-N and NO_3-N, nitrogen loading rate applied (NL), removed (NL_{rem}), and efficiency of removal of TN, nitrification and denitrification.

Cond.	Influent		Effluent				Efficiency		
	TKN (mg L^{-1})	NH_4-N (mg L^{-1})	TKN (mg L^{-1})	NO_3-N (mg L^{-1})	NL (Kg N m^{-3} d^{-1})	NL_{rem} (Kg N m^{-3} d^{-1})	TN (%)	Nitrif (%)	Denitrif (%)
1	25 ± 0.02	1.83 ± 0.01	3.00 ± 0.15	0.67 ± 0.11	0.18	0.15	85	88	97
2	24 ± 0.03	1.78 ± 0.01	7.68 ± 0.42	2.39 ± 0.20	0.17	0.10	59	69	86
3	20 ± 0.20	1.48 ± 0.01	5.17 ± 0.20	1.04 ± 0.02	0.24	0.12	69	75	93
4	20 ± 0.20	1.48 ± 0.01	11.12 ± 0.31	1.47 ± 0.03	0.24	0.07	38	46	84
5	21 ± 0.72	1.65 ± 0.02	6.30 ± 0.57	1.16 ± 0.29	0.19	0.12	65	70	92
6	20 ± 0.28	1.57 ± 0.01	6.02 ± 0.57	1.09 ± 0.15	0.18	0.12	65	71	92
7	21 ± 0.86	1.62 ± 0.01	6.31 ± 0.86	0.92 ± 0.20	0.19	0.12	65	70	92

Higher NL_{rem}, 0.15 kg N m^{-3} d^{-1}, was observed in Condition No. 1, with an HRT of 20 h and 3 h aeration (Table 4). The lowest NL_{rem} was obtained in Condition No. 4, with the lowest HRT and aeration time, 12 h and 1 h, respectively. Moura and coauthors [19] also found the highest NL_{rem} with the highest aeration time. This can be explained by the fact that an increase in the OD concentration improves nitrification.

Kinetic assays

Figure 5 shows the reactions of the concentrations of COD and TN at a 4 h cycle with different aeration times. The graph shows the tendency of a first-order reaction for COD removal with different aeration cycles in most of the assays that were performed (Figure 5a).

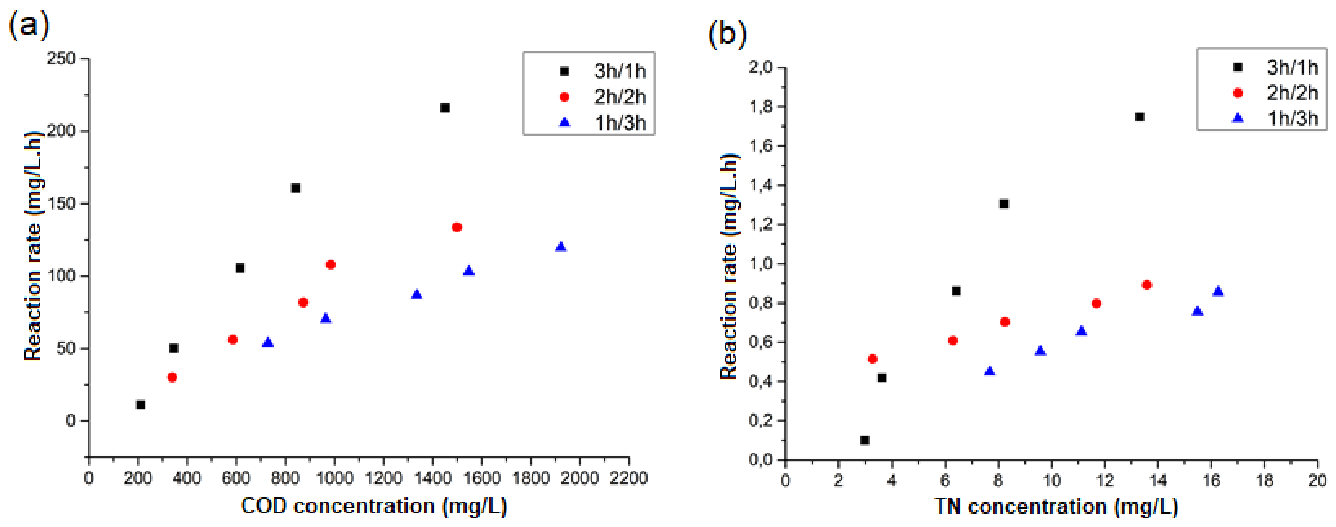

Figure 5. Graph showing (a) COD concentration and (b) TN concentration as a function of the reaction rate in 4 h cycles with different aeration times

The experiments that presented the highest values for the substrate removal rate constant (k_{COD}) were those that used the aeration cycle with the greatest availability of oxygen (3 h/1 h). In this case, the k_{COD} value was 0.1774 h^{-1}. The other cycles that were used (2 h/2 h and 1 h/3 h) produced lower k_{COD} values of 0.1203 h^{-1} and 0.0898 h^{-1} respectively. Although it contained substrate, the available oxygen was not sufficient. Okoli and coauthors [34] treated brewery effluent in a fluidized bed reactor and obtained a velocity constant value of 0.1251 h^{-1}. Borghei and coauthors [35] treated synthetic sugar-manufacturing wastewater in a fixed-bed aerobic biological reactor and obtained a velocity constant value of 0.6062 h^{-1}.

In relation to the total nitrogen consumption, first-order reactions were observed in the different aeration cycles in most of the assays. This is demonstrated in the graph of the reaction rate by TN concentration (Figure 5b). This graph shows that the aeration cycle with the greatest availability of oxygen (3 h/1 h) obtained a higher reaction rate constant than the other cycles (0.1723 h^{-1}). The nitrogen removal process requires an anoxic step, which occurred for 1 h each cycle, allowing the removal of nitrogen. The other cycles obtained k_{TN} values of 0.1176 h^{-1} and 0.0884 h^{-1} for 2 h/2 h and 1 h/3 h respectively. Niu and coauthors [36] treated synthetic wastewater in an up-flow anaerobic sludge blanket (UASB) reactor and obtained a reaction rate constant (k) of 0.0192 h^{-1}. These differences in k values might be attributed to differences in operational conditions and the type of wastewater [37].

In the present study, the cycles with shorter aeration times possibly did not have sufficient oxygen for nitrification to occur, as well as the formation of nitrate, which is the intermediate form of nitrogen used in the anaerobic phase (denitrification). Thus, even when these cycles had no oxygen supply for two or three hours there was no substrate to perform the conversion of the nitrate to gaseous nitrogen, and its consequent removal from the wastewater.

Competition for oxygen is another factor that may have interfered with the reaction rate in each of the types of cycle. In the cycle with the longest aeration time (3 h/1 h) the reaction rate was higher because oxygen was available for the bacteria that consumed the organic matter, and also to perform the aerobic conversion of nitrogen. In the other cycles, which had shorter aeration times, a competition for oxygen was initiated, damaging the conversion of nitrogen. The good nitrogen removal rates in the experiments, and the balance between the reactions (even if the COD/N ratio was high) occurred because there was no deficit in the supply of the carbon source for nitrogen removal by the SND process [38-40].

Although first-order kinetics did not provide the best agreement for all the assays, it was used to allow comparison between the results. Analyzing each assay, one-by-one, different kinetic orders were able to provide better adjustment; nevertheless, no global behavior was found.

pH and alkalinity

The pH values of the influent and effluent are shown in Table 5. The pH values in the brewery wastewater were not ideal for the nitrifying bacteria (7.0-9.0) and denitrifying bacteria (6.5-7.5). The autotrophic nitrification process consumes 7.14 mg of alkalinity (as $CaCO_3$) per mg of oxidized nitrogen. Thus, the pH was adjusted through the addition of 7.14 mg $CaCO_3$ per 1 mg of NTK, reaching the ideal range for nitrifying bacteria. The processes of ammonification and heterotrophic denitrification generate, as single processes, a total of 3.57 mg of alkalinity (as $CaCO_3$) per mg of ammonified/denitrified N [19].

Analyzing the alkalinity influent and effluent results measured during the reactor operation, it was possible to calculate the theoretical effluent alkalinity and to compare them both (Table 5). Table 5 shows that there was no difference between the theoretical and measured values, demonstrating that SND was the predominant reaction that occurred in the reactor.

Moura and coauthors [19], evaluated the efficiency of a reactor that used PU for biomass support, and recirculation and intermittent aeration to remove COD and TN from sewage. They also observed a similarity between the theoretical and measured alkalinity results. They assumed that this similarity might have been correlated to the occurrence of SND.

Table 5. Average concentrations of pH influent, pH effluent, influent alkalinity, theoretical effluent, and real alkalinity during the evaluated conditions.

Conditions	pH		Alkalinity		
			Influent	Effluent	
				Theoretical*	Measured
	Influent	Effluent	(mg $CaCO_3$ L^{-1})		
1	5.91 ± 0.01	8.47 ± 0.10	151 ± 0.5	132	125 ± 2
2	5.73 ± 0.25	8.24 ± 0.14	138 ± 0.5	96	100 ± 3
3	4.98 ± 0.05	7.78 ± 0.07	117 ± 0.5	89	95 ± 1
4	4.99 ± 0.05	7.39 ± 0.04	118 ± 0.5	67	69 ± 4
5	5.26 ± 0.01	8.64 ± 0.02	122 ± 0.6	90	96 ± 2
6	5.22 ± 0.02	8.69 ± 0.03	125 ± 0.6	93	97 ± 2
7	5.23 ± 0.03	8.59 ± 0.02	124 ± 0.5	91	94 ± 3

*Theoretical = influent alkalinity + (ammonified/denitrified N x 3.57) – (nitrified N x 7.14)

Influence of alkalinity correction

To verify the influence of the influent alkalinity on the reactor efficiency, Condition No. 1, the most efficient in terms of COD and TN removal, was repeated, but without alkalinity correction.

Table 6 demonstrates that without alkalinity correction, using 7.14 mg of $CaCO_3$ per 1 mg of TKN, effluent was obtained with the highest COD and TN concentrations, 566 ± 36 mg L^{-1} and 10 ± 0.60 mg L^{-1} respectively. With corrected alkalinity in the influent COD and NTK, concentrations of 209 ± 27 mg L^{-1} and 3 ± 0.15 mg L^{-1} respectively were obtained. With no influent alkalinity correction, the efficiency values of COD and TN removal declined to 78% and 43% respectively.

Table 6. Values for COD, TN, NH_4-N, NO_2-N, NO_3-N, pH and alkalinity for the best Condition, with and without alkalinity correction.

Parameters	No alkalinity correction		With alkalinity correction	
	Influent	Effluent	Influent	Effluent
COD (mg L^{-1})	2,683 ± 10	566 ± 36	2,682 ± 30	209 ± 27
TKN (mgL^{-1})	25 ± 0.20	10 ± 0.60	25 ± 0.02	3 ± 0.15
NH_4-N (mgL^{-1})	1.83 ± 0.01	0.00 ± 0.00	1.83 ± 0.01	0.00 ± 0.00
NO_2-N (mgL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NO_3-N (mgL^{-1})	0.00 ± 0.00	4.41 ± 0.78	0.00 ± 0.00	0.67 ± 0.11
pH	4.72 ± 0.12	5.16 ± 0.04	5.91 ± 0.01	8.47 ± 0.10
Alkalinity (mg$CaCO_3L^{-1}$)	26 ± 0.01	59 ± 2.88	151 ± 0.48	125 ± 2.53

MPN of nitrifying and denitrifying microorganisms

The MPN of the microorganisms was performed using the best experiment that was obtained, i.e., Condition No. 1. The MPN value for ammonia oxidizing bacteria (AOB) was 4.9×10^3 CFU 100 mL^{-1} and for nitrite oxidizing bacteria (NOB) it was 2.7×10^3 CFU 100 mL^{-1}. The MPN value for denitrifying bacteria was 3.3×10^6 CFU 100 mL^{-1}. Moura and coauthors [20] also observed higher MPN values for AOB than for NOB. The explanation for this is that ammonium oxidation liberates more energy than nitrite oxidation. The higher MPN values for denitrifying bacteria were because heterotrophic bacteria grow faster than nitrifying bacteria.

Oliveira and coauthors [42] compared the MPN of nitrifying and denitrifying bacteria in an anoxic/aerobic reactor and observed higher MPN for denitrifying bacteria. The authors explained that the cellular production of nitrifying bacteria is very low when compared to denitrifying bacteria because during nitrification 80% of the energy produced for the oxidation of inorganic compost is used for CO_2 fixation. Furthermore, for each atom of fixed carbon 35 moles of NH_4^+-N or 100 moles of NO_2^-—N are required.

CONCLUSION

The statistical analyses indicated that the removal of COD and TN were affected by the HRT (12, 16, and 20 h) and aeration time (1, 2, and 3 h, in cycles of 4 h). The lab-scale, batch, structured-bed reactor with recirculation and intermittent aeration system, using BioBob© carriers as biofilm media, was very effective for the simultaneous removal of COD and TN.

The best results were obtained in Condition No. 1 (20 h HRT and 3 h aeration), which used the highest HRT and aeration times. COD, TN, nitrification and denitrification efficiency of up to 92, 85, 88 and 97%, respectively were obtained, with effluent concentrations of 209 ± 28 mg COD L^{-1}; 3.00 ± 0.15 mg TKN L^{-1}; and 0.67 ± 0.11 mg NO_3-N L^{-1}. When using the same HRT, the higher the aeration time the higher the efficiency. In Condition No. 2 (20 h HRT and 1 h aeration) the effluent generated presented 729 ± 67 mg COD L^{-1}; 7.68 ± 0.42 mg TKN L^{-1}; and 2.39 ± 0.20 mg NO_3-N L^{-1}.

In Condition No. 3 (12 h HRT and 3 h aeration) effluent was generated with 615 ± 7 mg COD L^{-1}; 5.17 ± 0.20 mg TKN L^{-1}; and 1.04 ± 0.02 mg NO_3-N L^{-1}. However, in Condition No. 4 (12 h HRT and 1 h aeration) the effluent presented concentrations of 1,129 ± 3 mg COD L^{-1}; 11.12 ± 0.31 mg TKN L^{-1} and 1.47 ± 0.03 mg NO_3-N L^{-1}.

It was possible to conclude that, in the studied operational conditions, the single-batch reactor with Biobob© and intermittent aeration was able to remove COD and TN from brewery wastewater.

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