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Full Length Research Paper

Influence of recirculation rate on the performance of a combined anaerobic-aerobic reactor applied to the removal of carbon and nitrogen from poultry slaughterhouse wastewater

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The objective of this study was to evaluate a combined anaerobic-aerobic upflow fixed-bed reactor with liquid phase recirculation for the removal of nitrogen and organic matter from poultry slaughterhouse wastewater. The reactor performance was evaluated with a hydraulic retention time (HRT) of 11 h and three different recirculation rates (R=0.5; 1 and 2). The highest nitrogen removal efficiency value was obtained with an HRT of 11 h (6.8 h in the anaerobic zone and 4.2 h in the aerobic zone) and a recirculation rate of 2. In this condition, the total nitrogen removal efficiency was 69%, with effluent concentrations of 6 mg NH_4^+ L^{-1} and 12 mg NO_3^- L^{-1}. For all tested conditions, there was good chemical oxygen demand (COD) removal, with efficiency above 95%. The effect of dilution and the favoring of mass transfer caused by the increase in the recirculation rate positively influenced reactor performance.

Key words: Anaerobic degradation, nitrification, denitrification, combined reactor.

INTRODUCTION

Effluents from animal processing industries, such as slaughterhouses, after anaerobic processing have been used in many biological nitrogen removal studies in view of their eutrophic potential and the risks to aquatic life and human health due to the presence of nitrogen. Such studies often use a sequencing batch reactor (SBR) and aim to obtain information regarding the operational parameters that influence biological nitrogen removal,

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such as the initial concentration of ammonia nitrogen, the carbon to nitrogen ratio, the air flow and the cycle time (Andrade et al., 2010; Kummer et al., 2011; Dallago et al., 2012; Mees et al., 2011; Lima, et al., 2014; Lopes, et al., 2015).

In an SBR, nitrification and denitrification occur in a single reactor by alternating aerobic and anoxic periods, with sequential operations over time, divided into four steps: input, reaction, sedimentation and liquid discharge (Metcalf and Eddy, 2003). Based on the fact that effluents from anaerobic reactors have high NH_4^+-N/COD ratios, it is necessary to add an external source of organic carbon to achieve full denitrification. Methanol, ethanol and acetic acid are commonly used in the nitrogen removal process, but the use of external sources as electron donors to promote denitrification has limitations related to costs (Cervantes et al., 2001; Barana et al., 2013). The use of industrial effluents as carbon sources for denitrification is also an important strategy (Bernet et al., 1996; Mees et al., 2013). This requirement for an organic carbon source enables the use of endogenous electron donors from the anaerobic digestion, such as volatile organic acids, methane, ammonia or reduced sulfur compounds (Foresti et al., 2006), which are made possible in continuous systems.

A reactor consisting of anaerobic and aerobic compartments, constantly fed and with recirculation of the nitrified effluent to the anaerobic compartment, can enable the use of endogenous electron donors for the reduction of the nitrate into nitrogen gas from industrial effluents (Del Pozo and Diez, 2005; Araujo and Zaiat, 2009; Chan et al., 2012; Kreutz et al., 2014) and domestic sewage (Fazolo et al., 2007; Abreu and Zaiat, 2008; Oliveira Netto and Zaiat, 2012). There are few studies on the application of combined systems in the treatment of poultry slaughterhouse wastewater. Del Pozo and Diez (2005) used an anaerobic-aerobic reactor on a pilot scale with polystyrene foam as the support medium, with internal recirculation, for the treatment of poultry slaughterhouse wastewater and obtained organic matter and nitrogen removal efficiencies of 93 and 67%, respectively. However, the high internal recirculation associated with the air-lift effect caused mixing between the anaerobic and aerobic zones. Thus, most of the organic matter was removed aerobically, and only 12 to 34% of the total nitrogen (TN) removal was due to denitrification, limited by the dissolved oxygen concentration in the anaerobic zone, which was above 0.5 mg L^{-1} due to the mixing regime. In addition, most of the nitrogen removed was used in the synthesis of heterotrophic bacteria.

Araujo and Zaiat (2009) presented a new combined anaerobic-aerobic reactor configuration for the treatment of wastewater from the industrial processing of lysine. They observed that the best reactor operating condition was obtained with a hydraulic retention time of 35 h (21 h in the anaerobic zone and 14 h in the aerobic zone) and a recirculation ratio (r) of 3.5. Under that condition, the removal efficiencies of COD, total Kjeldahl nitrogen (TKN) and TN were 97, 96 and 77%, respectively.

In this context, the configuration in the present study was based on the reactor presented by Araujo and Zaiat (2009) and was designed to promote the recirculation of liquid from the aerobic zone to an intermediate anaerobic-anoxic zone, aimed at using the gases produced in the reactor during anaerobic digestion, such as nitrate reducers during the denitrification step, thus eliminating the need for providing an external source of organic carbon.

Therefore, the objective of this study was to evaluate the influence of effluent recirculation on the performance of a continuous anaerobic-aerobic upflow fixed-bed reactor for the removal of organic matter and nitrogen from poultry slaughterhouse wastewater.

MATERIALS AND METHODS

Combined anaerobic-aerobic reactor

The combined anaerobic-aerobic upflow fixed-bed reactor was manufactured out of an acrylic tube with an internal diameter of 93 mm and a length of 1,000 mm (Figure 1), as proposed by Araujo and Zaiat (2009). The reactor consisted of an inlet chamber (0.543 L volume), a reaction bed, an aeration chamber (0.407 L volume) and an outlet chamber (0.679 L volume). The reaction bed was divided into three compartments: anaerobic compartment I (Vu = 0.752 L), anaerobic compartment II (Vu = 2.199 L) and an aerobic compartment (Vu = 1.071 L). The working volume of the reactor was 5.651 L. Peristaltic dosing pumps were used to feed the reactor under a continuous upflow regime and to recirculate the effluent from the aerobic compartment to anaerobic compartment II. The oxygen was supplied to the aerated zone via a Big Air A360 aquarium pump, and the air was dispensed through porous rocks. The dissolved oxygen concentration was maintained above 2.0 mg L^{-1}.

Biomass inoculation and immobilization

Expanded clay with mean particle sizes ranging from 5 to 15 mm was used for immobilization of the biomass in anaerobic compartment I to minimize clogging of the reaction bed due to the presence of suspended solids in the wastewater and also to induce the adhesion of acidogenic bacteria throughout this material (Ortega et al., 2001). However, this compartment was not previously inoculated.

In the anaerobic II and aerobic compartments, polyurethane foam cubic matrices were used, with 1 cm edges, an apparent density of 23 kg/m^3 and a porosity of approximately 95%. The foam cubic matrices were embedded into sturdy plastic rings to support the material. To accelerate system startup, the polyurethane foam supports in the anaerobic II and aerobic compartments were previously inoculated. For the inoculation of anaerobic compartment II, an anaerobic sludge with 24.65 g VSS L^{-1} was used, with a biomass to wastewater ratio of 1:3. The support material remained immersed in the sludge for 24 h before it was introduced into the reactor. The system was operated in the anaerobic zone for 60 days, when the system exhibited stability in the reduction of COD and the formation of ammonia nitrogen. After that, and with the nitrifying biomass properly inoculated, the modules that made up

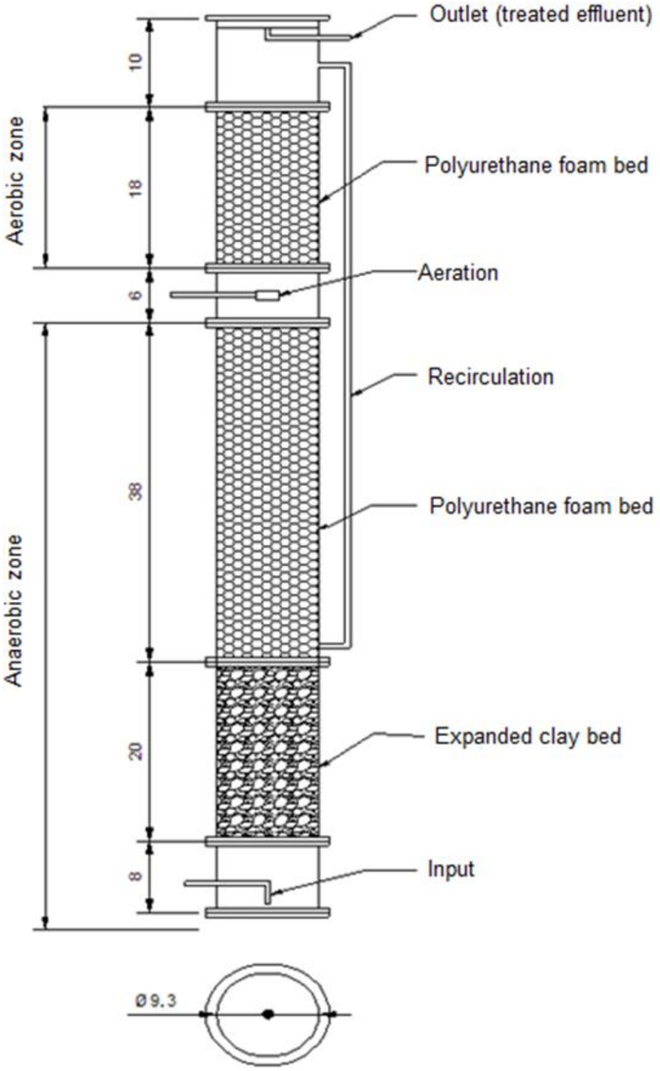


Figure 1. Diagram of the reactor. Araujo & Zaiat (2009).

the aerobic compartment were attached, and the system was operated under combined conditions for an additional 60 days.

Regarding the aerobic module, the support medium was inoculated with nitrifying sludge with 2.65 g VSS L^{-1} at a biomass to wastewater ratio of 1:3. This procedure was performed in a batch system in the laboratory with 24 h cycles over one week. When the support material exhibited significant nitrate formation, it was transferred to the reactor. Therefore, the adaptation stage of the biomass in the reactor lasted for 120 days and was divided into two steps; in the first 60 days, the reactor operated only with the anaerobic compartments, after which the aerobic compartment was then attached, and operations occurred under combined anaerobic-aerobic conditions. For both the anaerobic and aerobic adaptation stages, the hydraulic retention time (HRT) was 14 h with continuous input and no recirculation.

Substrate

The poultry slaughterhouse wastewater was sampled after the primary treatment (flotation system) to avoid problems caused by oil and grease deposition, such as obstruction, flotation, mass transfer problems and decreased methanogenic activity (Masse et al., 2003; Masse and Massé, 2005; Demirel et al., 2005; Saddoud and Sayadi, 2007).

After collection, the wastewater was stored into 2-L polyethylene terephthalate (PET) bottles and kept in a freezer until use. The wastewater characteristics are shown in Table 1.

Physico-chemical analyses

The system was monitored daily. The influent and effluent samples were characterized according to Standard Methods (APHA, 2005) for pH (potentiometric method 4500-H^+), total suspended solids (Method 2540-G), volatile suspended solids (Method 2540-E), TKN (5400-NT), total ammonia nitrogen (Method 4500-NH_4^+), and chemical oxygen demand (Method 5220-D); nitrate and nitrite were determined by flow injection analysis (Methods 4500-NO_3^- and 4500-NO_2^-). Alkalinity was determined by the method proposed by Ripley et al. (1986). The dissolved oxygen concentration was determined using an oximeter (model Orion 3 Star, Thermo Scientific, Beverly, MA, USA).

Experimental procedures

The reactor operation was divided into three stages (stages I, II and III) according to the recirculation conditions applied. The characteristics of each stage and the operating times are shown in Table 2. The HRT was 11 h, with 6.8 h in the anaerobic zone and 4.2 h in the aerobic zone. Thus, each stage was operated for at least 20 days after reaching a stationary state, defined by variations of less than 10% in three consecutive samples with respect to NH_4^+ removal, as proposed by Sahinkaya et al. (2011). The reactor was kept at a temperature of 30±0.1°C and controlled using a digital thermostat.

Data analysis

For data analysis, some equations were used, as described. The percentage of ammonia nitrogen was obtained with equation 1.

$$A(\%) = \left(1 - \frac{TKN_a - NH_{4e}}{TKN_e}\right) * 100 \qquad (1)$$

Where, TKN_a: total influent Kjeldahl nitrogen; TKN_e: total effluent Kjeldahl nitrogen; NH_{4e}: effluent ammonia nitrogen. The denitrification efficiency through recirculation (E_{DN}) was calculated according to Equation 2.

$$E_{DN} = \frac{N_{nitr} - N_e}{N_{nitr}} \qquad (2)$$

In Equation 2, N_{nitr} is the nitrified nitrogen concentration, and N_e is the sum of the concentrations of N-nitrite and N-nitrate present in the treated effluent. The removal efficiencies of TN, chemical oxygen demand (COD) and total suspended solids (TSS) were determined using equation 3:

$$S_e = \left(1 - \frac{E}{100}\right) * S_i \qquad (3)$$

Where, S_i: Influent substrate concentration (TN, COD or TSS); S_e: Effluent substrate concentration (TN, COD or TSS).

The upflow velocity was calculated according to Equation 4.

Table 1. Composition of the industrial wastewater used.

COD ($mg.L^{-1}$)	Total solids ($mg.L^{-1}$)	TKN ($mg.L^{-1}$)	NH_4^+-N ($mg.L^{-1}$)	NO_3^--N ($mg.L^{-1}$)	NO_2^--N ($mg.L^{-1}$)	Alkalinity ($mg.L^{-1}$)	pH
647±137	708±152	76±15	7.7±1.2	<0.05	<0.05	63±19	6.6±0.3

Note: Mean values and standard deviations of 16 analyzed samples.

Table 2. Operating time, upflow velocity (UFV), recirculation rate (Qr/Q), nitrogen loading rate (NLR) and organic loading rate (OLR) values for stages I, II and III, operated at a hydraulic retention time (HRT) of 11 hours and an inflow of 0.51 $L.h^{-1}$

Operating condition	Observation time (d)	UFV ($m.h^{-1}$)	Q_r/Q	NLR ($kg.N.m^{-3}.d^{-1}$)	OLR ($kg\ COD\ m^{-3}.d^{-1}$)
Stage I	22	0.112	0.5	0.134±0.010	1.137±0.112
Stage II	26	0.150	1	0.133±0.017	1.137±0.171
Stage III	25	0.225	2	0.157±0.007	1.329±0.133

$$UFV = \frac{Q_F + Q_R}{A} \quad (4)$$

Where, Q_F is the influent flow rate ($m^3\ h^{-1}$), Q_R is the recirculation flow rate ($m^3\ h^{-1}$) and A is the cross-sectional area of the reactor (m^2).

RESULTS AND DISCUSSION

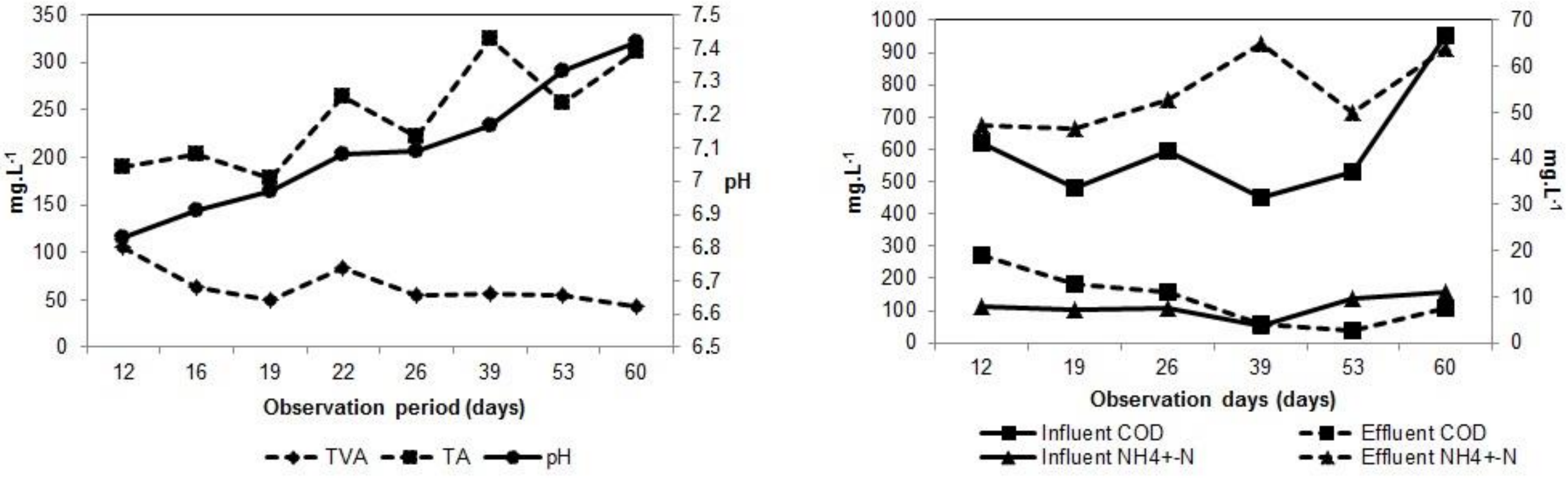
Biomass startup and adjustment in the reactor

After 60 days of operation in the anaerobic zone, in which only the two anaerobic compartments were in operation, the ammonification efficiency reached 75.4%, and the removal efficiencies of VSS and COD were 86 and 88%, respectively. Rajakumar and Meenambal (2008) evaluated an anaerobic hydroxide sludge blanket reactor, consisting of a support medium of PVC rings at the top of the reactor, with 5.4 L of usable volume applied to the treatment of poultry slaughterhouse effluents. The authors observed that the reactor startup was completed after 120 days of operation at temperatures ranging from 29 to 35°C, with a COD removal efficiency of 80%.

Figure 2 shows the profiles of the parameters pH, total alkalinity and total volatile acids in the final effluent, along with the COD and NH_4^+-N profiles for the influents and effluents of the reactor, during the startup period. The pH ranged from 6.4 to 7.4 and increased during the process. There was an increase in alkalinity due to ammonification and a conversion of COD to volatile organic acids, which led to sufficient system buffering capacity. The same trend was observed by Rajakumar et al. (2012). The TVA/TA ratio at the end of the period was 0.14; in Rajakumar et al. (2012), this ratio remained between 0.13 and 0.19, indicating system stability. The anaerobic reactor startup strategy was considered efficient, as it performed in a way that allowed considering that the system was adapted, enabling the startup of the aerobic compartment to proceed to the nitrification stage. The conversion of organic nitrogen into ammonia nitrogen was observed at the beginning of the operation of the reactor in the anaerobic module. At the end of the combined stage (anaerobic-aerobic), the conversion reached a mean efficiency of 70%.

Nitrifying activity was observed starting in the second week after the incorporation of the aerobic compartment, indicating effluents with NH_4^+-N values < 20 mg L^{-1} (Figure 3) after the fifth week, which is compatible with the current environmental law for the discharge of effluents into water bodies (Brasil, 2011). The conversion efficiency of NH_4^+-N into NO_2^--N + NO_3^--N was 47.1±11.1%, and approximately 20% of the influent nitrogen was removed from the system by assimilation. The TKN, NH_4^+-N and NO_3^--N parameter results analyzed during this test phase are shown in Table 3. Figure 3 shows the COD and NH_4^+-N results for the biomass startup and adaptation periods in the reactor. The COD varied in the input loads due to different batches but remained stable regarding the output values, reaching a removal efficiency of 92% at the end of this adaptation period.

Another indication of the onset of nitrification is the significant consumption of alkalinity. The mean values of total alkalinity and pH were, respectively, 73.1 mg L^{-1} and 6.5 in the influent and 9.4 mg L^{-1} and 5.4 in the effluent. The profiles of these indicators are shown in Figure 4. With the onset of nitrification, the inorganic carbon began to be consumed by chemoautotrophic microorganisms, with a consequent decrease in alkalinity to effluent values close to zero, which became a limiting factor in the nitrification process. This change was also observed in Oliveira Netto and Zaiat (2012). Because the combined



(a) **(b)**

Figure 2. (a) Total Alkalinity (TA), Total Volatile Acids (TVA) and pH (secondary scale) profiles for the reactor effluent; (b) COD and NH_4^+-N (secondary scale) profiles for the reactor influents and effluents.

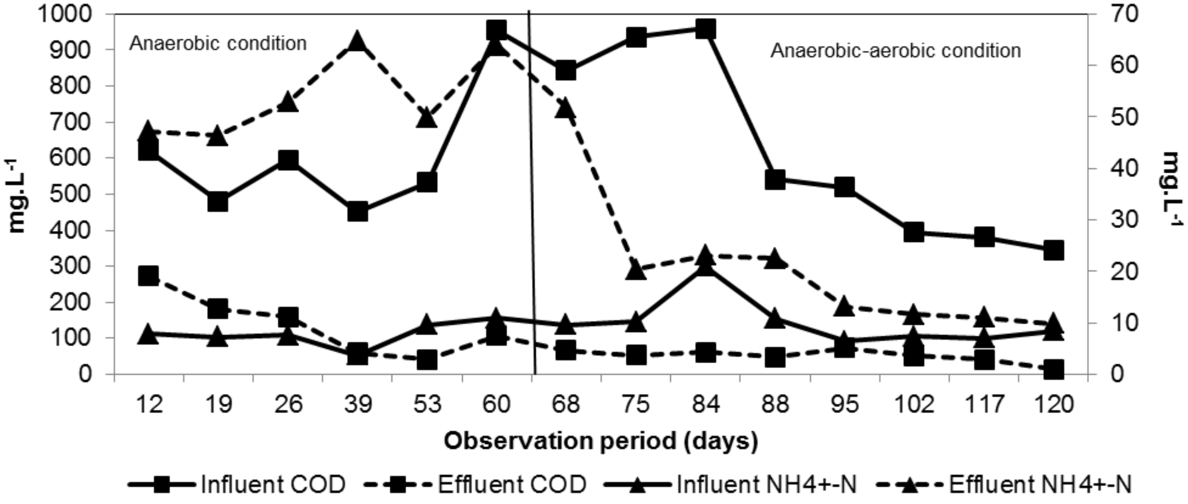


Figure 3. Organic matter concentration values expressed in terms of COD and ammonia nitrogen concentration (secondary scale) for the reactor influents and effluents for the biomass startup and adaptation periods.

Table 3. Results of the concentrations of the nitrogen compounds evaluated over time for the reactor influents and effluents during the system startup period.

Condition	Observation period (days)	Evaluated parameters Influent TKN (mg.L^{-1})	Effluent TKN (mg.L^{-1})	Influent NH_4^--N (mg.L^{-1})	Effluent NH_4^--N (mg.L^{-1})	Effluent NO_3^--N (mg.L^{-1})
Anaerobic	12	86.1	52.5	8.0	47.2	n.a.
	19	73.5	54.6	7.2	46.4	n.a.
	26	87.5	63.7	7.5	52.8	n.a.
	40	77.0	77.0	3.7	64.8	n.a.
	54	86.8	66.8	9.6	49.9	n.a.
	61	84.7	74.9	10.9	63.8	n.a.
Anaerobic-Aerobic	69	77.7	61.6	9.6	51.8	1.5
	76	95.2	28.0	10.2	20.3	13.3
	85	77.7	23.1	21.0	23.1	21.6
	89	81.2	32.9	10.8	22.9	21.6
	103	70.0	15.4	6.4	13.1	11.8
	110	52.5	14.7	7.3	11.6	12.7
	118	64.4	15.4	7.0	11.1	13.7
	120	73.5	23.8	8.4	13.6	23.4

n.a. – Not analyzed

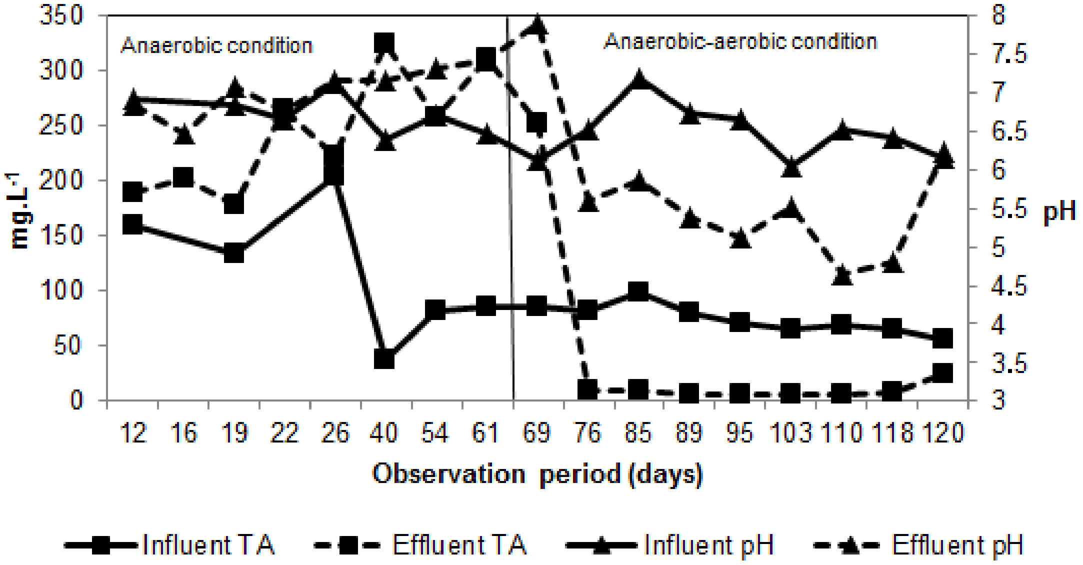


Figure 4. Input and output values of total alkalinity and pH (secondary scale) for the biomass startup and adaptation periods.

anaerobic-aerobic reactor operation promoted nitrification, conditions to promote denitrification were implemented in the next stage of the work. Thus, recirculation of the liquid phase from the aerobic compartment to anaerobic compartment II was contemplated to utilize the endogenous electron donors produced in the reactor during anaerobic digestion, such as nitrate reducers during the denitrification stage.

Nitrogen removal in the combined system with recirculation

After the adaptation period, the operation stages were initiated to maintain the HRT and recirculation conditions (Table 3) and were maintained for 73 days. The oxygen demand concentrations in the aerobic compartment for stages I, II and III, respectively, were 5.23±0.59, 4.96±0.66

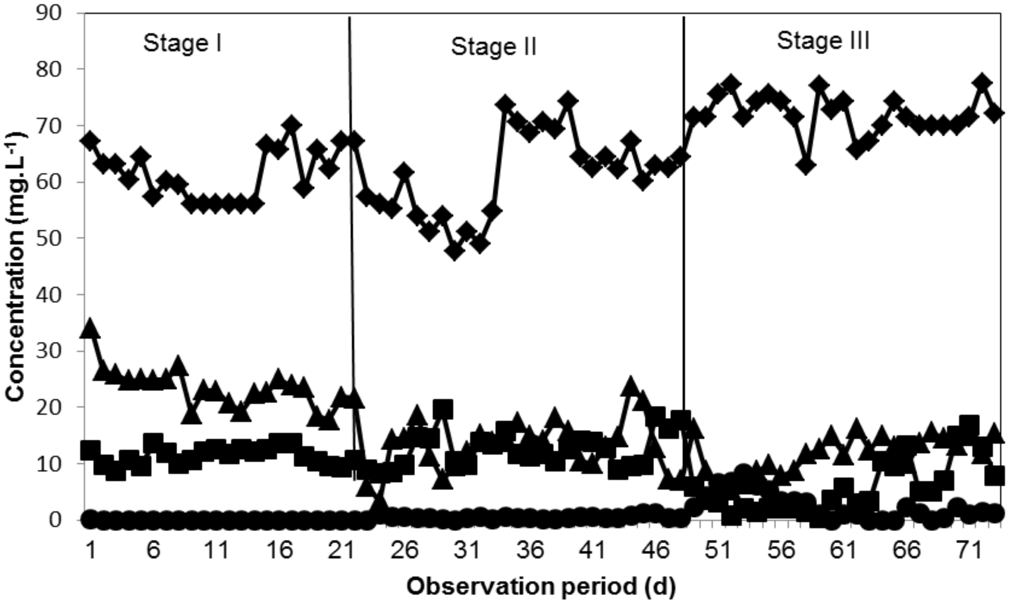


Figure 5. Temporal variations in the concentrations of influent TN (♦), effluent NH_4^+-N (■), effluent NO_3^--N (▲) and effluent NO_2^--N (●) during the three evaluated stages.

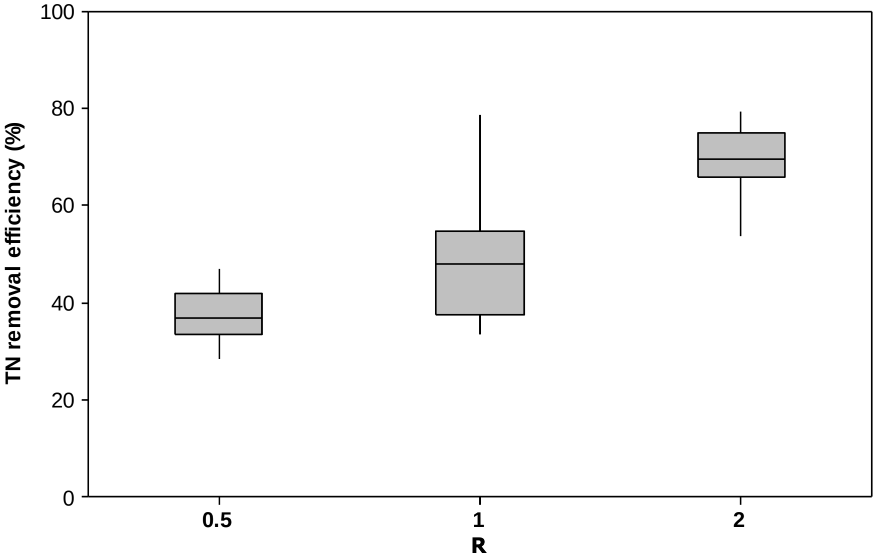


Figure 6. Box-plot of the distribution of TN removal efficiency at each stage, with maximum and minimum points, 1st and 3rd quartiles and medians.

and 4.69±0.21 mg L^{-1}. The oxygen needed for the oxidation of ammonia nitrogen is approximately 4.57 g O_2/g oxidized N-ammonia (Metcalf and Eddy, 2003). Oliveira Netto and Zaiat (2012) operated a combined anaerobic-aerobic fixed-bed reactor with liquid phase recirculation that was applied to the treatment of domestic sewage with an average concentration of dissolved oxygen of 5.7±0.7 mg L^{-1}. The temporal variations in the concentrations of TN, NH_4^+-N, NO_3^--N and NO_2^--N are illustrated in Figure 5. TN removal efficiencies of 37.6, 48.1 and 69.7% (Figure 6) were observed in stages I, II and III, respectively. The effective TN decrease was caused by the introduction of effluent recirculation, which aimed to direct the nitrate formed in the aerobic zone to anaerobic zone II of the reactor, allowing its denitrification by the use of electron donors

Table 4. Mean concentrations and standard deviations of total alkalinity (TA), bicarbonate alkalinity (BA) and pH evaluated in influents and effluents from stages I, II and III.

Parameter	Stage I		Stage II		Stage III	
	Influent ($mg.L^{-1}$)	Effluent ($mg.L^{-1}$)	Influent ($mg.L^{-1}$)	Effluent ($mg.L^{-1}$)	Influent ($mg.L^{-1}$)	Effluent ($mg.L^{-1}$)
TA	72	5	71	36	86	14
BA	32	0	28	24	32	6
pH	6.2	4.6	6.5	6.4	6.3	5.5

from the first compartment, such as organic matter, volatile organic acids and methane. The use of products from the anaerobic digestion as electron donors was considered satisfactory, and there was no external addition of carbon at any of the stages evaluated.

During stage III, where the highest TN removal efficiency occurred (69.7%), the effluent concentrations of NH_4^+-N, NO_3^--N and NO_2^--N were 5.6±4.7, 11±3 and 2.4±2.4 mg L^{-1}, respectively. In stages I and II, efficiency was decreased, mainly due to the reduction in the recirculation rate. Thus, it is noteworthy that the increase in internal recirculation of the nitrified effluent increased the amount of nitrate reduced to nitrogen gas and that there was a consequent decrease in the TN effluent concentration.

The increased recirculation rate promoted better mass transfer within the reactor. This improved transfer can be attributed to greater mixing due to the increase of the upflow velocity, which, according to Foladori et al. (2014), reduces channeling of preferential paths and improves the contact between the substrate and the bacterial biomass. In this experiment, the upward flow velocities were 0.113, 0.150 and 0.225 m h^{-1}, for stages I, II and III, respectively. Furthermore, an increased recirculation rate promotes liquid dilution within the reactor and may contribute to decrease the possible impacts caused by the variability of the input load (Jin et al., 2012).

Although stage III was operated with the highest input load (0.157±0.007 $kgNm^3 d^{-1}$) because of the variation in influent composition due to the fluctuation of the manufacturing process, it was at this stage that the system had the highest removal load: 0.139±0.01 kgm^3d^{-1}. This value is higher than that obtained by Oliveira Netto and Zaiat (2012), who operated a laboratory-scale anaerobic-aerobic fixed-bed reactor for the treatment of domestic sewage. When an HRT of 11.4 h and a recirculation rate of 1.5 were applied, the removed nitrogen load was 0.072 $kgm^3 d^{-1}$.

Araujo and Zaiat (2009) evaluated a combined anaerobic-aerobic fixed-bed reactor for the treatment of wastewater from lysine processing. They observed that under reactor operating conditions with a hydraulic retention time of 35 h (21 h in the anaerobic zone and 14 h in the aerobic zone) and recirculation ratios (r) of 0.5 and 1, the TN removal efficiencies were 42 and 54%, respectively. However, when these authors applied an r of 3.5 and an HRT of 35 h, the TN removal efficiency increased to 77%. Thus, they concluded that increasing the internal recirculation of treated effluent increases the amount of nitrate reduced to nitrogen gas through the denitrification in the anaerobic compartment of the reactor, with a subsequent decrease in the effluent TN concentration.

Although the TN removal was considered efficient in stage III, with the establishment of nitrification and denitrification, resupply of alkalinity to the medium by denitrification was not observed, as in this case, theoretically, for each 1 g of nitrate transformed to N_2, 3.57 g of alkalinity ($CaCO_3$) must be generated (Metcalf and Eddy, 2003). However, simultaneous nitrification and denitrification provided by the input of oxygen through recirculation prevented the quantification of alkalinity recovery in anaerobic compartment II. Thus, the effluent concentrations of total alkalinity, bicarbonate alkalinity and pH reached very low values (Table 4).

Alkalinity consumption resulted in decreased pH in the aerobic compartment in all evaluated stages. Despite the pH values being lower than 6.4, this parameter cannot be considered an inhibitor of nitrifying activity, but the absence of alkalinity was considered a limiting factor in the process. Throughout the reactor length, TA recovery in anaerobic compartment I was due to ammonification and conversion of volatile organic acids, which resulted in values of 247.3, 183.1 and 214.7 mg $CaCO_3$ L^{-1} for stages I, II and III, respectively. However, the TA recovery was not sufficient to meet the nitrification needs, and its deficit reached levels of 31, 34 and 35% for stages I, II and III, respectively. Furthermore, the industrial wastewater had low alkalinity concentrations of approximately 70 mg L^{-1}. For such cases, it is suggested that alkalinity be supplemented. However, it can be concluded that the higher recirculation rates provided the highest denitrification efficiencies (Table 5) and, theoretically, higher alkalinity recovery and TN removal.

Organic matter removal

The performance of the combined reactor relative to COD removal can be seen in Table 6. The most removal occurred in the first compartment due to anaerobic digestion; therefore, the COD removal was constant, and

Table 5. Effluent concentrations of nitrogen in the nitrite and nitrate forms (N_e), denitrification efficiencies (E_{DN}) and TN removal efficiencies (E_{TN}), in stages I, II and III.

Stage	**Parameters**		
	N_e (mg.L^{-1})	**E_{DN} (%)**	**E_{TN} (%)**
I	23.5	36.7	37.4
II	13.2	60.9	48.7
III	14.2	72.1	69.4

Table 6. Mean concentrations and standard deviations of COD evaluated in influents and effluents and the removal efficiencies of COD in stages I, II and III.

Stage	**Influent COD (mg.L^{-1})**	**Effluent COD (mg.L^{-1})**	COD Removal (%)
I	521±51	14±3	97±1
II	521±78	15±6	97±1
III	609±61	27±12	96±2

recirculation did not affect this parameter. Such values are relatively higher than those found by Kreutz et al. (2014) when evaluating a chambered anaerobic-aerobic fixed-bed reactor with a recirculation of the liquid phase of 0.5. The usable volume of that reactor was 4.75 L, and for an HRT of 11 h, the authors obtained a COD removal efficiency of 59%. However, the system was fed with cattle slaughterhouse wastewater and had an organic load of 2.288 kg.m^3.d^{-1}, higher than those applied in the present work.

Asadi et al. (2012) evaluated the simultaneous removal of carbon and nutrients from industrial wastewater in a single aerobic-anoxic upflow bioreactor with a sludge blanket. Optimized removals of 80 and 50% were obtained for COD and TKN, respectively, when the HRT was 12 h and the aeration time was 40 to 60 min/h. The low concentrations of total suspended solids in the effluent, approximately 26, 48 and 42 mg L^{-1}, with adequate efficiency in the removal of this parameter, indicated that the increased upflow velocity, due to recirculation, did not cause biomass detachment throughout the evaluation period.

Conclusions

The evaluated system was efficient in removing organic matter for all conditions applied and in removing TN, found with the application of an HRT of 11 h and a recirculation rate of 2. The effect of dilution and the favoring of the mass transfer caused by the increase in the recirculation rate contributed to better reactor performance. Therefore, the this system with recirculation of the liquid phase is advantageous for the treatment of effluent from poultry slaughterhouses, as it promotes the secondary treatment and removal of nutrients in a single reactor with no need for an external carbon source.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Abreu SB, Zaiat M (2008). Desempenho de reator anaeróbio-aeróbio de leito fixo no tratamento de esgoto sanitário. Eng. Sanit. Amb. 13(2):181-188.

Andrade L, Kummer ACB, Fazolo A, Damasceno S; Hasan SDM, (2010). Influência de nitrogênio amoniacal e vazão de ar no processo de nitrificação, etapa de tratamento de efluente de abatedouro de peixe. Eng. Agríc. 30:160-167.

APHA, AWWA, WEF (2005). Standard Methods for the Examination of Water and Wastewater, 21th ed. APHA, Washington DC.

Araujo Jr MM de, Zaiat M (2009). An Upflow Fixed-bed Anaerobic–Aerobic Reactor for Removal of Organic Matter and Nitrogen from L-Lysine Plant Wastewater. Can. J. Civil Eng. 36:1085-1094.

Asadi A, Zinatizadeh AA, Sumathi S (2012). Simultaneous removal of carbon and nutrients from an industrial estate wastewater in a single up-flow aerobic/anoxic sludge bed (UAASB) bioreactor. Water Res. 46:4587-4598.

Barana AC, Lopes DD, Martins TH, Pozzi E, Damianovic MHRZ, Del Nery V, Foresti E (2013). Nitrogen and organic matter removal in an intermittently aerated fixed-bed reactor for post-treatment of anaerobic effluent from a slaughterhouse wastewater treatment plant.

J. Environ. Chem. Eng. 1:453-459.

Bernet N, Habouzit F, Moletta R (1996). Use of an industrial effluents a carbon source for denitrification of a high-strength wastewater. Appl. Microb. Biotechnol. 45:92-97.

Brasil (2011). Ministério Do Meio Ambiente. Conselho Nacional Do Meio Ambiente – Conama, 2011. Resolução 430: classificação dos corpos de água. Brasília: Ministério do Meio Ambiente.

Cervantes FJ, De La Rosa D, Gómez J (2001). Nitrogen removal from wastewaters at low C/N ratios with ammonium and acetate as eléctron donos. Bioresour. Technol. 79:165-170.

Chan YJ, Chong MF, Law CL (2012). Start-up, steady state performance and kinetic evaluation of a thermophilic integrated anaerobic–aerobic bioreactor (IAAB). Bioresour. Technol. 125:145-157.

Dallago RC, Gomes SD, Mees JBR, Assis TM, Hasan SDM, Kunz A (2012). Nitrification and denitrification of a poultry slaughterhouse wastewater according to cycle time and ammoniacal nitrogen concentration using surface response. Int. J. Food Agric. Environ. 10:856-860.

Del Pozo R, Diez V (2005). Integrated anaerobic–aerobic fixed-film reactor for slaughterhouse wastewater treatment. Water Res. 39:1114-1122.

Demirel B, Yenigun O, Onaya TT (2005). Anaerobic treatment of dairy wastewaters: a review. Process Biochem. 40:2583-2595.

Fazolo A, Foresti E, Zaiat M (2007). Removal of Nitrogen and Organic Matter in a Radial-Flow Aerobic-Anoxic Immobilized Biomass Reactor Used in the Posttreatment of Anaerobically Treated Effluent. Appl. Biochem. Biotechnol. 142:4-51.

Foladori P, Ruaben J, Ortigara ARC, Andreottola G (2014). Batch feed and intermittent recirculation to increase removed loads in a vertical subsurface flow filter. Ecol. Eng. 70:124-132.

Foresti E, Zaiat M, Vallero M (2006). Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges. Rev. Environ. Sci. Biotechnol. 5:3-19.

Jin R, Yang G, Ma C, Yu J, Zhang Q, Xing B (2012). Influence of effluent recirculation on the performance of Anammox process. Chem. Eng. J. 200-202:176-185.

Kreutz C, Passig FH, Carvalho KQ, Mees JBR, Gomes SD (2014). Desempenho de um reator anaeróbio-aeróbio e estudo cinético de remoção de matéria orgânica de efluente bovino. Eng. Agríc. 34:341-351.

Kummer ACB, Andrade A, Gomes SD, Fazolo A; Hasan SDM, Machado F (2011). Tratamento de efluente de abatedouro de tilápia com adição de manipueira na fase anóxica. Eng. Agríc. 31:150-157.

Lima AN de, Gomes BM, Gomes SD, Carvalho KQ de, Christ D (2014). Application of response surface methodology to study the biological removal of nitrogen from effluent of cattle slaughterhouse in a sequencing batch reactor. Eng. Agríc. 34:363-371.

Lopes CL, Mees JBR, Sene L, Carvalho KQ de, Christ D, Gomes SD (2015). Effect of cycle time and airflow in biological nitrogen removal from poultry slaughterhouse wastewater using sequencing batch reactor. Eng. Agríc. 35:567-577.

Masse L, Massé DI (2005). Effect of soluble organic, particulate organic, and hydraulic shock loads on anaerobic sequencing batch reactors treating slaughterhouse wastewater at 20°C. Process Biochem. 40:1225-1232.

Masse L, Massé DI, Kennedy KJ (2003). Effect of hydrolysis pretreatment on fat degradation during anaerobic digestion of slaughterhouse wastewater. Process Biochem. 38:1365-1372.

Mees JBR, Gomes SD, Hasan SDM, Gomes BM, Vilas Boas MA (2013). Nitrogen removal in a SBR operated with and without pre-denitrification: effect of the carbon:nitrogen ratio and the cycle time. Environ. Technol. 34:1-18.

Mees JBR. Damasceno S, Vilas Boas M, Gomes BM, Passig FH (2011). Kinetic behavior of nitrification in the post-treatment of poultry wastewater in a sequential batch reactor. Eng. Agríc. 31:954-964.

Metcalf L, Eddy H (2003). Wastewater engineering: treatment, disposal and reuse. 3rd ed. New York: McGraw Hill.

Oliveira Netto AP de, Zaiat M (2012). Treatment of Domestic Sewage in an Anaerobic–Aerobic Fixed-bed Reactor with Recirculation of the Liquid Phase. Clean Soil Air Water 40:965-971.

Ortega FS, Rocha KM, Zaiat M, Pandolfelli VC (2001). Aplicação de espumas cerâmicas produzidas via ‘‘gelcasting’’ em biorreator para tratamento anaeróbio de águas residuárias. Cerâmica 47:199-203.

Rajakumar R, Meenambal T (2008). Comparative study on start-up performance of HUASB and AF reactors treating poultry slaughterhouse wastewater. Int. J. Environ. Res. 2:401-410.

Rajakumar R, Meenambal T, Saravanan PM, Ananthanarayanan P (2012). Treatment of poultry slaughterhouse wastewater in hybrid upflow anaerobic sludge blanket reactor packed with pleated poly vinyl chloride rings. Bioresour. Technol. 103:116-122.

Ripley LE, Boyle WC, Converse JC (1986). Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. J. Water Pollut. Control Fed. 48:406-411.

Saddoud A, Sayadi S (2007). Application of acidogenic fixed-bed reactor prior to anaerobic membrane bioreactor for sustainable slaughterhouse wastewater treatment. J. Hazard. Mater. 149:700-706.

Sahinkaya E, Dursun N, Kilic A, Demirel S, Uyanik S, Cinar O (2011). Simultaneous heterotrophic and sulfur-oxidizing autotrophic denitrification process for drinking water treatment: Control of sulfate production. Water Res. 45:6661-6667.