



## Prediction of total nitrogen removal in a structured bed reactor for secondary and tertiary treatment of sanitary sewage

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### ABSTRACT

In this study, a structured bed reactor with recirculation and intermittent aeration was used to predict total nitrogen (TN) removal. The influent was a mixture of untreated sewage and effluent from an upflow anaerobic sludge blanket (1:1). The reactor was operated with continuous flow ( $Q$ ), recirculation of twice the entered flow ( $2Q$ ), at a temperature of 30°C. Seven tests were performed, with aeration times of 60, 75, and 90 min in 180-min cycles, and hydraulic retention time (HRT) of 8, 10, and 12 h. With an HRT of 8 h and aeration of 60 min, it was possible to obtain 88% TN removal and an effluent with 18 mg L<sup>-1</sup> of chemical oxygen demand (COD) and 4.3 mg L<sup>-1</sup> of TN. The study showed that the mathematical model obtained was predictive for TN removal and nitrification efficiency. It was found that the SBRRIA was efficient for both secondary and tertiary treatment of sanitary sewage, and removing COD and TN.

*Keywords:* Structured bed reactor; Intermittent aeration; Factorial design; Modeling; Nitrification; Denitrification

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### 1. Introduction

In most wastewater treatment plants (WWTPs), the chemical oxygen demand (COD) and nutrient removal operations occur in different reactors. Previous studies have shown that it is possible to remove COD and total nitrogen (TN) in a single reactor [1–3].

Studies have also shown that the use of a structured bed reactor with recirculation and intermittent aeration (SBRRIA) allows the occurrence of simultaneous nitrification and denitrification (SND) and the removal of COD in a single environment [2–5]. This is possible due to the gradient

of oxygen concentration in the biofilm. The presence of oxygen in the outer layers enables aerobic, autotrophic, nitrifying bacteria from the *Nitrosomonas* and *Nitrobacter* genera to develop, which converts the ammoniacal nitrogen (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) [6,7]. In the deeper layers, with the presence of nitrate and the absence of oxygen, there is an anoxic environment that is suitable for the development of heterotrophic denitrifying bacteria, which convert the nitrate to gaseous nitrogen and use organic matter as a carbon source, thereby reducing the COD content [1,6,8].

The advantages of removing COD and TN in a single reactor include the following: lower costs regarding the

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implementation of the system is because it is possible to perform the secondary and tertiary treatments in a single compartment; lower costs in relation to the acquisition of chemical reagents is because part of the alkalinity consumed during nitrification is returned to the system during denitrification; lower carbon consumption is because heterotrophic, denitrifying bacteria will consume the COD that is not used by autotrophic nitrifying agents; lower energy consumption is because facultative, heterotrophic bacteria can use nitrate as an oxygen source, thereby reducing the need for aeration; and less generation of sludge [2,7,9].

In Brazil, practically all the studies regarding the feasibility of WWTPs include anaerobic treatment as the main process [10]. In Paraná state, situated in the south of Brazil, 96% of the 203 WWTPs use upflow anaerobic sludge blanket (UASB) reactors for the main process, followed by anaerobic filter, stabilization pond, or activated sludge reactors [11]. The use of UASB reactors as one of the stages in WWTPs has been consolidated because of improvements in the mass and energy balance, due to the reduction in the generation of sludge, and also because of the production of methane, which is potentially usable as energy in urban WWTPs.

These configurations make it possible to remove COD, but they do not remove nitrogen, which can cause the eutrophication of water bodies and also disease in humans, making it difficult to reuse effluent [6,12,13].

The aim of this study is to evaluate the simultaneous removal of COD and TN, as well as the efficiency of an SBRRIA treating an influent mixture of untreated sewage/effluent from a UASB (50%, v/v). In addition, a mathematical model is proposed and validated, which estimates the efficiency of TN removal in an SBRRIA operated under different hydraulic retention times (HRTs) and aeration times.

## 2. Materials and methods

### 2.1. Sanitary sewage

Sanitary sewage, which was predominantly domestic sewage from a WWTP with an average inlet flow of  $230 \text{ L s}^{-1}$  located in the south of Brazil and serving a population of approximately 100,000 inhabitants, was used for the experiment. Two sewage streams were used: sewage collected after preliminary treatment, which was composed of grid and sandbox; and sewage already treated by a UASB. The influent used in this experiment consisted of a mixture containing 50% (v/v) of each stream. This mixture was used to provide a carbon source for the denitrification process, thereby avoiding the use of an external carbon source.

A previous study demonstrated that using an influent mixture of untreated sewage/effluent from a UASB, with mixtures containing 25/75, 50/50, 75/25, and 100/0 (v/v), respectively, there was no statistical difference in the efficiency of TN removal, which varied between  $50\% \pm 15$  and  $64\% \pm 18$  [14]. Consequently, the use of a 50% (v/v) influent mixture was proposed because of the possibility of using the methane produced by a UASB for energy production, as well as duplicating the capacity of a WWTP when implementing a SBRRIA reactor.

### 2.2. Reactor

The reactor was cylindrical, 80.0 cm in height, with an internal diameter of 14.5 cm and a useful volume of 8.6 L. It was filled with 13 cylinders of polyurethane foam arranged longitudinally for growth and biomass fixation, with a height of 63.0 cm and diameter of 2.0 cm (Fig. 1). The density of the foam was  $22 \text{ g L}^{-1}$  and the porosity was 90.

For this experiment, the reactor was operated for 240 d at a constant temperature of  $30^\circ\text{C}$  and submitted to intermittent aeration. During the aerated phases, the dissolved oxygen (DO) content was between 2.0 and  $2.5 \text{ mg L}^{-1}$ . To keep the system aerated, three common aquarium air compressors (ACQUA FLUX series A 01) were used, connected to air outlet hoses provided with porous stones. The aerators were connected to a timer in order to use different aeration times.

The reactor was equipped with a recirculation system with a flow rate equal to twice the inlet flow, the output was at the top of the reactor (A4), and the entrance was at the base (A2). The reactor was fed from the bottom (A1), and the effluent was collected at the top (A3) (Fig. 1).

### 2.3. Physicochemical analysis

To follow up the experiment, pH, COD, total Kjeldahl nitrogen (TKN), ammoniacal nitrogen ( $\text{N-NH}_4^+$ ), nitrite ( $\text{N-NO}_2^-$ ), and nitrate ( $\text{N-NO}_3^-$ ) analyses were performed according to methods described in American Public Health Association [15]. The alkalinity analyses were performed according to Ripley et al. [16].

### 2.4. Experimental design and statistical analysis

The ranges of HRT and aeration (factors) in relation to the removal of COD and TN (response variables) were determined using a  $2^k$  factorial design, where  $k$  is the number of factors, and 2 is the number of factor levels, with three central points. The conditions used to evaluate the TN, COD removal, and nitrification and denitrification efficiency are set out in Table 1. The design composed seven tests, of which

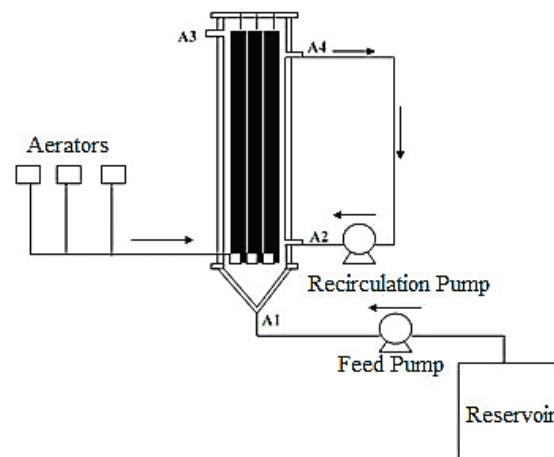


Fig. 1. Schematic representation of the structured bed reactor with intermittent aeration: (A1) influent feed, (A2) recirculation entrance, (A3) effluent output, and (A4) recirculation output. Source: the authors.

Table 1  
Independent variables (factors), HRTs and aeration times (coded and real values), and days of operation

Tests	Coded		Real			Days of operation
	HRT	Aeration	HRT (h)	Aeration (min)	No aeration (min)	
1	1	1	12	90	90	30
2	1	-1	12	60	120	20
3	-1	1	8	90	90	23
4	-1	-1	8	60	120	27
5	0	0	10	75	105	24
6	0	0	10	75	105	31
7	0	0	10	75	105	25

four of the tests were at levels +1 and -1; there were three replicates in the central point.

The mathematical modeling of the process was performed using response surface methodology (RSM) coupled with multiple linear regression. For this purpose, a linear model was used to fit the experimental data. The generalized model used in the RSM is expressed in Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \quad (1)$$

where  $Y$  is the predicted response and  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_{12}$  represent the regression coefficient ( $\beta_0$  represents the term for the intersection;  $\beta_1$  and  $\beta_2$  represent the linear effects; and  $\beta_{12}$  represents the interaction effect). The proposed models only predicted values between the intervals of the independent, tested variables, and the values used in the equation should be coded.

The statistical significance of the proposed models was evaluated by analysis of variance (ANOVA). The terms that did not show significant difference were removed and were re-fitted to the determined significant parameters ( $p < 0.05$ ); the 3D-response surfaces were then constructed. The goodness of fit of the models were evaluated by  $p$  (lack of fit), the determination coefficient ( $R^2$ ), and their adjusted  $R^2$ . The normality of residual analysis of all the models was tested by using the Kolmogorov–Smirnov test.

The data obtained from the experimental design were analyzed using Statistica 13.2 (TIBCO Software Inc., Palo Alto, CA, USA) software. The data were first checked for normality by the Shapiro–Wilk test, and ANOVA tests were then performed. Subsequently, when there was difference between the samples, Tukey's test was performed at a level of 95%, using R software for Windows, in order to verify the difference between the tests.

All the results were obtained using the apparent steady state condition. The apparent steady state was considered when the efficiency data for TN removal did not vary by more than 10% during 5 d. The results obtained in the transient state were not considered.

### 2.5. Preparation of predictive mathematical model and validation of the same

After the experimental design of the seven tests, which was determined by the factorial planning, a response surface was generated and a mathematical model was developed

to predict the TN removal and nitrification efficiency using different HRTs and aeration times. The model was considered predictive when it did not have lack of fit.

To validate the results obtained from the modeling, the following four complementary experiments were performed with different factors: (a) HRT of 12 h (+1) and aeration of 90 min (+1); (b) HRT of 12 h (+1) and aeration of 60 min (-1); (c) HRT of 10 h (0) and aeration of 75 min (0); and (d) HRT of 8 h (-1) and aeration of 60 min (-1). Each of these complementary experiments was conducted for 15 d.

## 3. Results and discussion

### 3.1. Reactor efficiency

The reactor used in this study had the same configuration as that proposed by Moura et al. [3]. This reactor has been operated for more than 700 d in other studies using the same type of substrate, sewage mixture, and UASB effluent, but with different operating conditions in terms of HRT and aeration times.

Table 2 presents the COD, TKN, and  $N-NH_4^+$  results in relation to the reactor effluent and influent. The results regarding  $N-NO_2^-$  and  $N-NO_3^-$  refer only to the effluent, because their presence in the influent was not detected.

The final effluent quality in terms of COD ranged from  $18 \pm 11$  to  $59 \pm 19$  mg L<sup>-1</sup>. These values meet the standard for effluent discharge for COD for this type of WWTP, which is 125 mg L<sup>-1</sup>. The high efficiency of COD removal in all the tests can be explained by the fact that reactors operating with SND systems can remove COD in the aerobic phase, by heterotrophic, aerobic bacteria, and in the anoxic phase, by facultative, denitrifying, heterotrophic bacteria [9,17,18]. Moura et al. [3] used an SBRRIA to remove COD and TN from synthetic sewage; they were able to remove 85%–89% of COD, with HRTs ranging from 8–12 h. Santos et al. [5] also evaluated an SBRRIA to remove COD and TN by using different influent C/N ratios; they found no statistical difference between the COD removal tests, which was above 94%. Liu and Wang [19] worked with another reactor model utilizing intermittent aeration; they also evaluated the removal of COD and TN and did not find a difference in COD removal when comparing HRTs of 22 and 24 h and aeration/no aeration times of 2 h/2 h, 1.5 h/1.5 h, 1 h/2 h, and 1.5 h/3 h.

Statistical analysis showed that, within the studied levels, the factors of aeration and HRT were not statistically

Table 2

Mean values of reactor influent and effluent in relation to COD, TKN, N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>2</sub><sup>-</sup>, and N-NO<sub>3</sub><sup>-</sup>

Tests	Variables (mg L <sup>-1</sup> )							
	Influent			Effluent				
	COD	TKN	N-NH <sub>4</sub> <sup>+</sup>	COD	TKN	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>2</sub> <sup>-</sup>	N-NO <sub>3</sub> <sup>-</sup>
1	166 ± 47	45 ± 7	22 ± 2	38 ± 28	7 ± 5	1 ± 0.9	0.2 ± 0.4	2.0 ± 0.1
2	165 ± 54	60 ± 17	30 ± 5	36 ± 24	8 ± 4	1 ± 0.8	1.0 ± 0.8	2.0 ± 0.2
3	138 ± 20	36 ± 9	24 ± 1	37 ± 24	3 ± 2	0.6 ± 0.9	0.7 ± 0.4	2.0 ± 0.1
4	242 ± 120	37 ± 11	28 ± 3	18 ± 11	1 ± 1	1 ± 0.9	0.8 ± 0.4	2.0 ± 0.1
5	206 ± 14	74 ± 16	46 ± 5	54 ± 20	4 ± 1	3 ± 2.0	2.0 ± 1.0	2.0 ± 0.2
6	166 ± 38	53 ± 12	36 ± 12	59 ± 19	5 ± 3	4 ± 6.0	2.0 ± 1.0	2.0 ± 0.1
7	189 ± 109	45 ± 13	18 ± 8	43 ± 13	4 ± 3	0.4 ± 0.4	1.0 ± 0.6	2.0 ± 0.1

significant at a 95% probability level in terms of the removal of COD, because the *p*-value was greater than 0.05 for all variables. In other words, the HRT and aeration levels that were studied did not interfere with COD removal.

The efficiency values regarding TN removal, nitrification, and denitrification, as well as the COD/N ratio obtained throughout the experiment, are shown in Table 3. It can be seen that there was highly efficient nitrification and denitrification in all the tests, and therefore a high level of TN removal. The worst results regarding the efficiency of TN removal, between 76% and 81% in Tests 1 and 2, were obtained with an HRT of 12 h.

This highly efficient level of nitrification may have been due to low concentrations of easily biodegradable organic matter in the effluent, because it was composed of a mixture of UASB effluent and primary sewage. Low C/N ratios decrease the competition for DO between aerobic heterotrophic and autotrophic nitrifying microorganisms, favoring the activity of the latter [1,5,20–22]. A study of the molecular biology of a constructed wetland revealed that ammonia-oxidizing bacteria predominated when the C/N ratio was lower than six [23]. Gong et al. [24] investigated SND in sanitary sewage and observed the growth of larger amounts of autotrophic biomass than heterotrophic biomass; they attributed this to the low C/N ratio of the influent, which was 2.94.

It was possible to observe the high denitrification efficiency, which was higher than 90%, in all the tests. This resulted in low concentrations of nitrite and nitrate in the effluent, which were 2 ± 1 and 2 ± 0.2 mg L<sup>-1</sup>, respectively (Tables 2 and 3).

The high level of TN removal obtained in all the tests can be explained by the following factors:

- The fact that the polyurethane support medium allowed the occurrence of aerobic and anoxic environments at different depths of the foam cylinders (SND). Thus, in the outermost regions, where OD diffusion takes place, nitrification occurs because oxygen is present and, therefore, aerobic nitrifying bacteria are present. In the deeper regions, where oxygen cannot diffuse, the action of the denitrifying, facultative, heterotrophic bacteria prevails, which consume the COD and reduce nitrite and nitrate to gaseous nitrogen.
- The COD/TN ratio of the influent, which was between 2.8 ± 0.3 and 4.5 ± 3, was theoretically insufficient for

denitrification to occur. Thus, the high levels of denitrification efficiency may be due to the carbon derived from endogenous metabolism because of the high sludge retention time (SRT) [24,25]. Gong et al. [24] evaluated SND in sanitary sewage in an MBBR and noted that with a long SRT there was an increase in the population of nitrifying bacteria and a considerable decrease in aerobic heterotrophs. Thus, the heterotrophic, denitrifying bacteria used the organic matter more intensely than the heterotrophic aerobic bacteria.

- It is also possible that the removal of nitrogen occurred because of anammox processes. According to Henze et al. [26], a C/N ratio ranging from 3.5 to 4.5 is necessary for heterotrophic denitrification to occur. The COD/N influent varied from 2.8 to 4.5. Considering that part of the organic matter was oxidized by heterotrophic bacteria, the COD/N that remained was less than necessary for heterotrophic denitrification to occur. Consequently, nitrogen removal could have occurred due to bacteria that performed anammox activity. Barana et al. [2] used an SBRRIA to treat UASB effluent from a slaughterhouse and found anammox activity in the COD/N of the influent that varied from 2.2 to 2.6.
- The occurrence of the nitrite denitrification pathway. Bernat et al. [27] suggested that the use of nitrites as electron acceptors was responsible for the high level of denitrification that they obtained, with COD/N = 3–4. Ramos et al. [28] observed denitrification, with COD/N = 2.5. Torà et al. [29] observed levels of consumed COD/N = 3.0 for nitrite denitrification and COD/N = 3.9–4.3 for nitrate denitrification, corroborating the possibility of the role of this pathway in this study.

Table 4 presents the statistical values for the analysis of TN removal and nitrification efficiency, at a significance level of 90%, *p* ≤ 0.1. At the levels that were tested, the aeration and HRT factors were significant in the removal of TN because the *p*-value was less than 0.1. When considering a *p*-value less than 0.1, we are of the opinion that 90% of the results which were obtained can be attributed to the factors of aeration and HRT. The regression coefficient for HRT and aeration at –3.75 and –2.25, respectively, shows that the higher these values, the lower the efficiency in terms of TN removal (Fig. 2(a)).

Barana et al. [2] used an SBRRIA to treat UASB effluent from a chicken slaughterhouse. An HRT of 24 h and the

following different aeration times were used: continuous aeration; 4 h aerated and 2 h without aeration; 2 h aerated and 1 h without aeration; 1.5 h aerated and 1.5 h without aeration; and 1 h aerated and 2 h without aeration. The best TN removal results (62%) were obtained with the shortest aeration time; 1 h with aeration and 2 h without aeration. Under these operational conditions, and with an influent COD of 418 mg L<sup>-1</sup>, TKN of 169 mg L<sup>-1</sup>, and N-NH<sub>4</sub><sup>+</sup> of 112 mg L<sup>-1</sup>, effluent was obtained with COD of 22 mg L<sup>-1</sup>, TKN of 6.4 mg L<sup>-1</sup>, N-NH<sub>4</sub><sup>+</sup> of 6.4 mg L<sup>-1</sup>, and N-NO<sub>3</sub> of 58 mg L<sup>-1</sup>.

Correa et al. [30] studied sanitary sewage using the same model of reactor as this study, but with an HRT of 16 h and intermittent aeration of 4 h aeration and 2 h without aeration; they observed nitrification efficiency of 70% ± 21% and denitrification of 70% ± 20%.

Wosiack et al. [4] used the same reactor model but the effluent that was treated was from a pet food industry; they used 24 h HRT and continuous aeration and obtained TN removal rates of 79%, 100% nitrification, and 79% denitrification. The aforementioned authors used these results to verify the existence of aerobic and anoxic regions in the PU foam, which allowed denitrification to occur, even with continuous aeration.

Table 3

TN removal, nitrification, and denitrification efficiency with average and standard deviation, and the influent COD/TN ratio

Test	TN (%)	Nitrification (%)	Denitrification (%)	COD/TN
1	76 ± 4.3	83 ± 4.9	93 ± 1.9	3.6 ± 1.1
2	81 ± 5.5	87 ± 4.0	93 ± 2.5	2.8 ± 1.2
3	84 ± 6.9	91 ± 4.5	91 ± 3.8	3.8 ± 0.4
4	88 ± 3.5	96 ± 2.1	91 ± 1.5	6.5 ± 2.6
5	86 ± 8.0	94 ± 6.9	93 ± 2.4	2.7 ± 0.3
6	82 ± 3.6	91 ± 3.4	91 ± 3.1	3.1 ± 0.7
7	84 ± 4.7	90 ± 4.3	93 ± 1.8	4.2 ± 3.0
Average	83 ± 5.2	90 ± 4.3	92 ± 2.4	3.8 ± 1.3

Table 4

Regression coefficients, standard error, ±90% confidence limits, and significance of the generated regression models for TN removal and nitrification efficiency

Factors	Regression coefficient	Standard error	t-value	p	-90% Confidence limit	+90% Confidence limit
TN removal (%)—R <sup>2</sup> = 0.85; R <sup>2</sup> adj = 0.78; p (model) = <0.05						
Mean/Interc.	83.00	0.69	119.53	<0.01	81.52	84.48
(1) HRT	-3.75	0.92	-4.08	0.02	-5.71	-1.79
(2) Aeration	-2.25	0.92	-2.45	0.07	-4.21	-0.29
p (lack of fit)	0.59					
p (normality of residues)	>0.20					
Nitrification (%)—R <sup>2</sup> = 0.83; R <sup>2</sup> adj = 0.75; p (model) = <0.05						
Mean/Interc.	90.29	0.82	109.81	<0.01	88.53	92.04
(1) HRT	-4.25	1.09	-3.91	0.02	-6.57	-1.93
(2) Aeration	-2.25	1.09	-2.07	<0.10	-4.57	0.07
p (lack of fit)	0.46					
p (normality of residues)	>0.20					

RSM, coupled with multiple linear regression, generated a mathematical equation with a correlation between HRT and aeration time, and TN removal (Fig. 2(a)) (Eq. (2)). The proposed model did not have lack of fit ( $p = 0.59$ ) and presented an adjusted  $R^2$  value of 0.78, indicating that the mathematical model was predictive for TN removal. According to Granato et al. [31],  $R^2$  values above 0.7 can be considered as “good”. The lack of fit  $p$ -values that are greater than 0.05 showed that the developed model was adequate for predicting the response [32].

$$\text{TN (\%)} = 83.00 - 3.75 \times \text{HRT} - 2.25 \times \text{Aeration} \quad (2)$$

The statistical values of the factors of HRT and aeration time also showed significance in relation to nitrification, at a level of 90%,  $p \leq 0.1$ . The RSM generated a mathematical equation with a correlation between HRT and aeration time in relation to nitrification (Fig. 2(b)) (Eq. (3)). The model presented an adjusted  $R^2$  value of 0.75 and did not show lack of fit ( $p = 0.46$ ), indicating that the mathematical model was predictive for nitrification. It was not possible to correlate the response denitrification with the factors of HRT and aeration time.

$$\text{Nitrification} = 90.29 - 4.25 \times \text{HRT} - 2.25 \times \text{Aeration} \quad (3)$$

Besides RSM was not used to optimize the experiment, but to help to obtain an equation that could predict it, it can be seen in Figs. 2(a) and (b) that lower HRT and aeration time studied, better was nitrification and TN removal.

### 3.2. Validation of the predictive mathematical model

Considering the future design of this type of reactor, it was possible to predict possible average values for TN removal efficiency using Eq. (1).

Thus, at this stage of the experiment, the reactor was operated with the following four complementary conditions: HRT = 12 h (+1) with aeration = 90 min (+1);

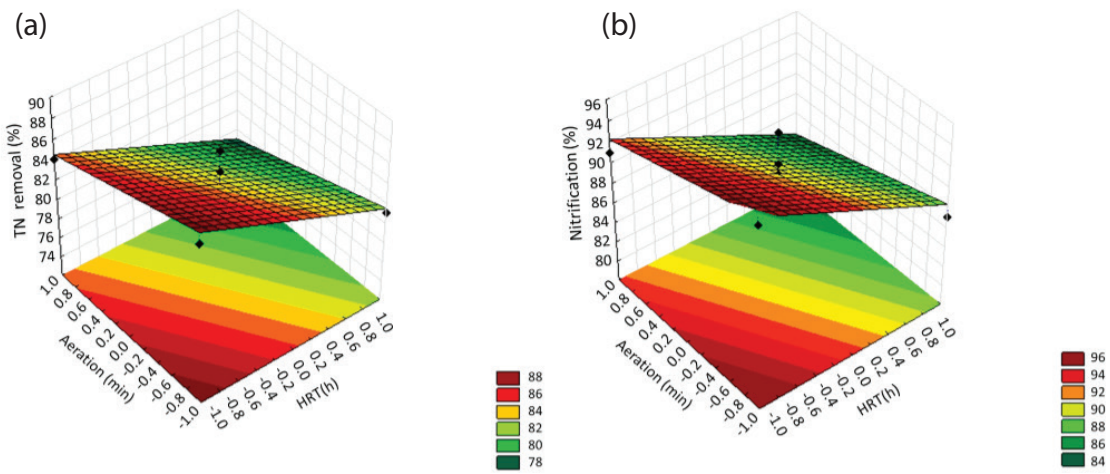


Fig. 2. Response surface plots (coded values) to show the effects of the factors of aeration and HRT on (a) TN removal and (b) nitrification efficiency.

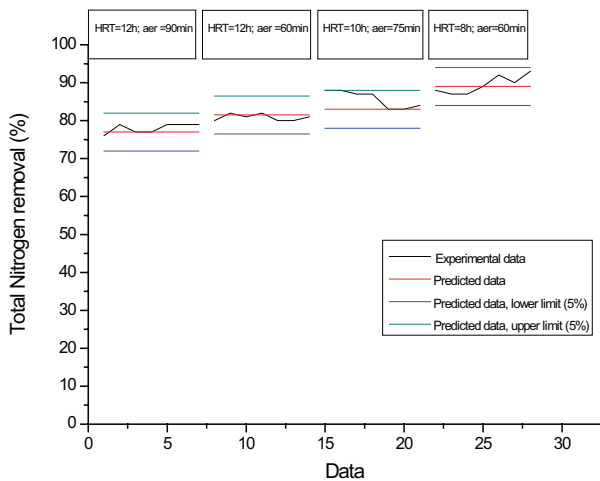


Fig. 3. Total nitrogen removal (%) responses with different levels of factors: predicted and experimental data.

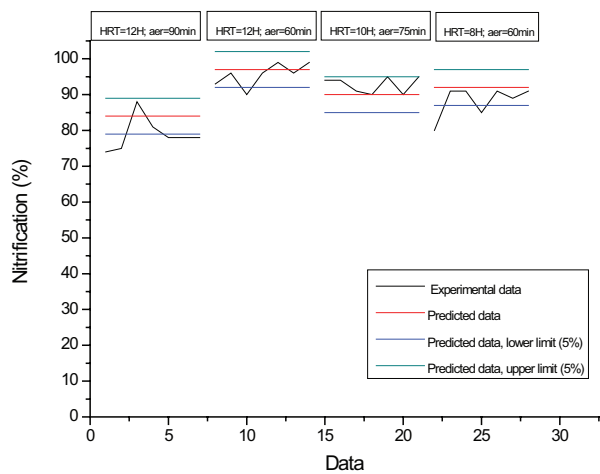


Fig. 4. Nitrification efficiency (%) response with different levels of factors: predicted and experimental data.

HRT = 12 h (+1) with aeration = 60 min (−1); HRT = 10 h (0) with aeration = 75 min (0); and HRT = 8 h (−1) with aeration = 60 min (−1) during 60 d. It was verified if the results obtained experimentally were consistent with the predicted ones (Figs. 3 and 4).

Fig. 3 shows that all the experimental results performed according to the predicted data for TN removal.

In terms of nitrification efficiency (Fig. 4), almost all the experimental results fitted with the predicted data. Therefore, the mathematical model that was generated was predictive and can be used to anticipate results without the need for practical experimentation, resulting in savings in time and costs regarding reagents.

#### 4. Conclusion

The SBRRIA was efficient in removing COD and TN from sewage. The effluent COD was between 18 and 59 mg L<sup>−1</sup>. The statistical analyses indicated that the COD removal was not affected by HRT (8, 10, and 12 h) and aeration time (60, 75, and 90 min in cycles of 180 min). The TN removal efficiency varied between 76% and 88%. The denitrification efficiency showed no significant difference between the tests. It was possible to predict TN removal and nitrification by using the mathematical model generated by RSM. Using the equations that were generated to predict results may help in future studies, saving time, and experimental costs.

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#### References

[1] X. Zhang, H. Zhang, C. Ye, M. Wei, J. Du, Effect of COD/N ratio on nitrogen removal and microbial communities of CANON process in membrane bioreactors, *Bioresour. Technol.*, 189 (2015) 302–308.

- [2] A.C. Barana, D.D. Lopes, T.H. Martins, E. Pozzi, M.H.R.Z. Damianovic, V. Del Nery, E. Foresti, 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 (2013) 453–459.
- [3] R.B. Moura, M.H.R.Z. Damianovic, E. Foresti, Nitrogen and carbon removal from synthetic wastewater in a vertical structured-bed reactor under intermittent aeration, *J. Environ. Manage.*, 98 (2012) 163–167.
- [4] P.A. Wosiack, D.D. Lopes, M.H.R.Z. Damianovic, E. Foresti, D. Granato, A.C. Barana, Removal of COD and nitrogen from animal food plant wastewater in an intermittently-aerated structured-bed reactor, *J. Environ. Manage.*, 154 (2015) 145–150.
- [5] C.E.D. Santos, R.B. Moura, M.H.R.Z. Damianovic, E. Foresti, Influence of COD/N ratio and carbon source on nitrogen removal in a structured-bed reactor subjected to recirculation and intermittent aeration (SBRRRIA), *J. Environ. Manage.*, 166 (2016) 519–524.
- [6] Y. Liu, H. Shi, L. Xia, H. Shi, T. Shen, Z. Wang, G. Wang, Y. Wang, Y. Study of operational conditions of simultaneous nitrification and denitrification in a Carrousel oxidation ditch for domestic wastewater treatment, *Bioresour. Technol.*, 101 (2010) 901–906.
- [7] M. Seifi, M.H. Fazaelpoor, Modeling simultaneous nitrification and denitrification (SND) in a fluidized bed biofilm reactor, *Appl. Math. Modell.*, 36 (2012) 5603–5613.
- [8] B. Virdis, S.T. Read, K. Rabaey, R.A. Rozendal, Z. Yuan, J. Keller, Biofilm stratification during simultaneous nitrification and denitrification (SND) at a biocathode, *Bioresour. Technol.*, 102 (2011) 334–341.
- [9] C.S.A. Canto, J.A.D. Rodrigues, S.M. Ratusznei, M. Zaiat, E. Foresti, Feasibility of nitrification/denitrification in a sequencing batch biofilm reactor with liquid circulation applied to post-treatment, *Bioresour. Technol.*, 99 (2008) 644–654.
- [10] C.A.L. Chernicharo, *Anaerobic Reactors*, Series: Biological Wastewater Treatment, Vol. 4, IWA, London, 2016, P. 188.
- [11] B.Z.L. Ross, Escuma de reatores anaeróbios tratando esgotos domésticos em escala real: produção, caracterização e proposição de parâmetros para seu gerenciamento, PhD thesis (Doutorado em Engenharia de Recursos Hídricos e Ambiental), Universidade Federal do Paraná, Curitiba, 2015, p. 163.
- [12] H.R. Powley, H.H. Durr, A.T. Lima, M.D. Krom, P. Van Cappellen, Direct discharges of domestic wastewater are a major source of phosphorus and nitrogen to the Mediterranean Sea, *Environ. Sci. Technol.*, 50 (2016) 8722–8730.
- [13] M. Kumar, L. Lin, Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal-strategies and issues, *J. Hazard. Mater.*, 178 (2010) 1–9.
- [14] A.C. Wendling, Remoção simultânea de matéria orgânica e nitrogênio total em reator de leito estruturado com aeração intermitente tratando efluente composto por esgoto bruto e efluente de RALF, MSc thesis (Mestrado em Engenharia Sanitária e Ambiental), Universidade Estadual de Ponta Grossa, Ponta Grossa, 2017, p. 67.
- [15] American Public Health Association (APHA), AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington D.C., USA, 2005.
- [16] L.E. Ripley, W.C. Boyle, J.C. Converse, Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes, *J. Water Pollut. Control. Fed.*, 58 (1986) 406–411.
- [17] I. Schmidt, O. Sliemers, M. Schmid, E. Bock, J. Fuerst, J.G. Kuenen, M.S.M. Jetten, M. Strous, New concepts of microbial treatment processes for the nitrogen removal in wastewater, *FEMS Microbiol. Rev.*, 27 (2003) 481–492.
- [18] Y. Chiu, L. Lee, C. Chang, A.C. Chao, Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor, *Int. Biodeterior. Biodegrad.*, 59 (2007) 1–7.
- [19] G. Liu, J. Wang, Achieving advanced nitrogen removal for small flow wastewater using a baffled bioreactor (BBR) with intermittent aeration, *J. Environ. Manage.*, 199 (2017) 222–228.
- [20] A. Asadi, A.A. Zinatizadeh, M. Van Loosdrecht, H. Younesic, Nitrogen removal by ANAMMOX and simultaneous nitrification–denitrification (SND) processes in a novel single airlift bioreactor, *RSC Adv.*, 6 (2016) 74367–74371.
- [21] S. Luostarinen, S. Luste, L. Valentin, J. Rintala, Nitrogen removal from on-site treated anaerobic effluents using intermittently aerated moving bed biofilm reactors at low temperatures, *Water Res.*, 40 (2006) 1607–1615.
- [22] K. Hanaki, C. Wantawin, S. Ohgaki, Effects of the activity of heterotrophs on nitrification in a suspended-growth reactor, *Water Res.*, 24 (1990) 289–296.
- [23] W. Zhi, G. Ji, Quantitative response relationships between nitrogen transformation rates and nitrogen functional genes in a tidal flow constructed wetland under C/N ratio constraints, *Water Res.*, 64 (2014) 32–41.
- [24] L. Gong, L. Jun, Q. Yang, S. Wang, B. Ma, Y. Peng, Biomass characteristics and simultaneous nitrification–denitrification under long sludge retention time in an integrated reactor treating rural domestic sewage, *Bioresour. Technol.*, 119 (2012) 277–284.
- [25] G.H. Chen, H. Ozaki, Y. Terashima, Endogenous denitrification in biofilm, *Water Sci. Technol.*, 26 (1992) 523–534.
- [26] M. Henze, G.H. Kristensen, R. Strube, Rate-capacity characterization of wastewater for nutrient removal processes, *J. Water Sci. Technol.*, 29 (1994) 101–107.
- [27] K. Bernat, D. Kulikowska, M. Zielinska, A. Cydzik-Kwiatkowska, I. Wojnowska-Baryla, Nitrogen removal from wastewater with low COD/N ratio at low oxygen concentration, *Bioresour. Technol.*, 110 (2011) 4913–4916.
- [28] C. Ramos, M.E. Suarez-Ojeda, J. Carrera, Denitrification in an anoxic granular reactor using phenol as sole organic carbon source, *Chem. Eng. J.*, 288 (2016) 289–297.
- [29] J.A. Torà, J.A. Baeza, J. Carrera, J.A. Oleszkiewicz, Denitrification of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources, *Chem. Eng. J.*, 172 (2011) 994–998.
- [30] C.Z. Correa, K.V.M.C. Prates, C. Azevedo, D.D. Lopes, E.A. Rodrigues, G. Peterlini, A.C. Barana, Comportamento da remoção de matéria orgânica (DQO) e nitrogênio amoniacal em um reator de leito estruturado operado sob aeração intermitente, *Sci. Plena.*, 12 (2016) 1–10.
- [31] D. Granato, V.M.A. Calado, B. Jarvis, Observations on the use of statistical methods in food science and technology, *Water Res. Int.*, 55 (2014) 137–149.
- [32] A.A.F. Zielinski, C.W.I. Haminiuk, T. Beta, Multi-response optimization of phenolic antioxidants from white tea (*Camellia sinensis* L. Kuntze) and their identification by LC–DAD–Q–TOF–MS/MS, *LWT–Food Sci. Technol.*, 65 (2016) 897–907.