Evaluating the potential of a hybrid baffled reactor for simultaneous organic matter and nitrogen removal of dairy wastewater

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Abstract

This work designed and assessed the performance of a structured-bed hybrid baffled reactor (SBHBR) with anaerobic/anoxic chambers, treating different dairy wastewater. The anoxic chambers in SBHBR were exposed to intermittent aeration for the simultaneous removal of organic matter and total nitrogen (TN) under a low COD/TN ratio. The hydraulic retention time (HRT) in SBHBR was 48h, with 16.3h in the anoxic zone, where intermittent aeration was implemented, consisting of 60 minutes of aeration and 30 minutes without aeration. The COD/TN ratios tasted were 2.1 ± 0.6, 0.84 ± 0.5, and 0.35 ± 0.1 in the inlet of the anoxic chambers. The SBHBR provided COD removal efficiencies above 90% in all experimental stages. The relevant results achieved in this research regarding carbon and nitrogen removal efficiencies were obtained in stage III. The SBHBR achieved a TN removal efficiency of 82.3 ± 11.4% during this stage. The nitrification and denitrification efficiencies were 85.9 ± 17% and 85.2 ± 9%, respectively, resulting in the anoxic zone TN removal efficiency of 74.6 ± 14.7% with a C/N ratio of 0.35 ± 0.1. Stoichiometric calculations based on nitrogen removal and the C/N ratio required by the denitrification process were used to corroborate the activity of bacteria that perform the anammox pathways as their main mechanism.

Introduction

The dairy industry is present worldwide and generates large amounts of wastewater from milk production, manufacturing processes, and derivatives. The content of wastewater depends on the industrial process; they usually consist of high concentrations of organic matter and nitrogenous compounds (SHAMS et al., 2018). Dairy wastewater treatment can be achieved through physical, chemical, and biological treatment. Therefore, each treatment has different advantages and disadvantages depending on the characteristics of the effluent, best available technology, jurisdictions, and regulations (Bustillo-Lecompte et al., 2016; RIVAS et al., 2010; STRUK-SOKOLOWSKA et al., 2018)

Typically, dairy effluents are treated in anaerobic reactors because this technology suits effluents with a high concentration of organic matter. The high conversion of organic matter in anaerobic reactors generates smaller volumes of sludge, in addition to the possibility of recovering valuable by-products, such as the generation of biogas, fertilizers, and water for agricultural reuse (DABROWSKI et al. 2017; DOMINGUES et al. 2015; MENEGASSI et al. 2020). Despite the aforementioned advantages, anaerobic reactors cannot wholly remove carbon and nitrogen contents, such as ammonia, from the wastewater; on account of that, a post-treatment is frequently needed (FORESTI et al., 2006).

The conventional biological nitrogen removal occurs in sequential reactions of autotrophic nitrification and heterotrophic denitrification under aerobic and anoxic conditions, respectively. In the nitrification process, ammonium (NH_4^+-N) is oxidized to nitrite (NO_2^--N) by ammonia-oxidizing bacteria (AOB), then nitrite-oxidizing bacteria (NOB) oxidize NO_2^--N to nitrate (NO_3^--N). During denitrification, heterotrophic facultative bacteria use organic matter as electron donors and the oxidized forms of nitrogen (NO_2^--N or NO_3^--N) as electrons acceptor, reducing to gaseous nitrogen (N_2).

Among the possibilities for anaerobic effluent post-treatment, the processes that combine nitrification and denitrification in a single operational unit proved to be a remarkable alternative to improve the efficiency of simultaneous removal of organic matter and nitrogen (CAI et al., 2019; MOURA et al., 2012). Hybrid systems are generally assumed to play a role in the main contributions of ammonia and nitrite oxidation (LO et al. 2010). Furthermore, previous studies mainly demonstrated that a structured bed reactor with intermittent aeration allows the growth of different species of microorganisms (SANTOS et al., 2016, ALMEIDA et al., 2018; CORREA et al., 2018; MOURA et al., 2018; CHEN et al., 2021). The polyurethane foam used as a support medium for microbial growth has a low cost. It can increase solid retention time (SRT) due to high porosity and good mechanical resistance. Moreover, the dissolved oxygen (DO) gradient formed in the biofilm may allow nitrifying microorganisms to attach to the external layer, where the DO concentration is higher. In contrast, denitrifying bacteria can grow inside the biofilm due to lower DO concentration (WIJFFEL and TRAMPER, 1995).

Baffled reactors can be established as viable and optimized alternatives for removing organic matter and nitrogen. In addition to the intrinsic advantages of compartmentalization (such as resistance to organic and hydraulic loads), the baffled reactor allows combining suspended and fixed biomass to establish anaerobic and anoxic environments. Thus, nitrification and denitrification processes have been successfully carried out in hybrid baffled reactors operated with continuous aeration and recirculation of nitrified effluent to the denitrification compartment (BUSTILLO-LECOMPTE et al., 2013; LEYVA-DÍAZ et al., 2015).

This study aimed to develop a simple continuous feed bioreactor under intermittent aeration and without recirculation to achieve efficient removal of organic carbon and nitrogen by treating dairy wastewater at a low COD/TN ratio using an endogenous carbon source as an electron donor. This perspective offers essential insights into different biological processes integrated into the structured-bed hybrid baffled reactor (SBHBR) under anaerobic conditions and oxygen limitations in distinct environments.

Material And Methods

Operational setup

Figure 1 shows the structured-bed hybrid baffled reactor (SBHBR). The bench-scale SBHBR was made of acrylic with dimensions of 50 cm in length, 25 cm in width, and 30 cm high (5 cm headspace) and consisted of five equal-volume chambers of 6.45 liters each. The working volume was 25.5L, correlating to a bed porosity of 60%.

(Fig. 1 inserted here)

The feeding was performed by a four-channel peristaltic pump (Gilson Mini Plus) distributed equidistantly along the width of the bioreactor. Chambers C1 and C2 were operated with suspended sludge, and chambers C3, C4, and C5 were operated with part of the sludge attached. Eighty-eight strip polyurethane (PU) foam structures were used as material support in each chamber. The PU structure was fixed by a stainless wire fence and attached to the extremities of the chambers. An aerator (BOYU-S510) supplied oxygen, with a maximum flow rate of 2.0 L min^{-1}, into compartments C4 and C5. Diffuser porous stick stones of 10 cm were placed at the bottle of C4 and C5 to improve air distribution in these chambers.

Inoculation procedure and operational conditions

The hydraulic retention time (HRT) of the SBHBR was 48 hours, calculated from the work volume. Therefore, based on working volume, the anaerobic and anoxic condition was 31.7 and 16.3 hours, respectively. The temperature was maintained at 37 ± 1.8ºC in a temperature-controlled chamber. The first three chambers were anaerobic, and the last two were destined for continuous or intermittent aeration with periods of 60 minutes of aeration and 30 minutes of non-aeration (stages I, II, and III). The anaerobic chambers (C1, C2, and C3) were inoculated with a mixture (50% by volume) of anaerobic sludges pulled out of an operating Upflow Anaerobic Sludge Blanket (UASB) reactor treating poultry slaughterhouse effluent (Dacar, Tietê, SP, Brazil) and an anaerobic sequencing batch reactor treating dairy effluent (PUSP-P, Pirassununga, SP, Brazil). The anoxic chambers (C4 and C5) were inoculated with biomass taken from a Bardenpho system treating glutamate monosodium effluent (Ajinomoto, Limeira, SP, Brazil). The inoculation was made according to Zaiat et al. (1994) in chambers C3, C4, and C5. The reactor was operated with continuous aeration until steady state verification in the start-up period, which meant constant oxidizing of ammonium present in the bulk liquid (variation lower than 1.0% in nitrification) and consistent bicarbonate alkalinity production. This period was considered the reactor start-up. During the three employed experimental stages, the reactor was fed with three different dairy wastewater sources under the same intermittent aeration condition to evaluate the response of SBHBR under the different sources of dairy wastewater. The inoculation, start-up period, and stage I were conducted by treating dairy wastewater collected from a dairy treatment plant of the University of São Paulo (USP), located at Pirassununga, SP, Brazil. Stage II: treating simulated dairy wastewater from milk pasteurization (laboratory-made solution in Table 1) and stage III: treating dairy wastewater collected in a dairy effluent plant of an industry Jamava, located at Santa Cruz da Conceição, SP, Brazil.

Table 1

Laboratory-made solution to simulate wastewater from milk pasteurization

Components	Concentration
Skimmed milk	2.7g $COD.L^{-1}$
Nitric acid	0,39 $ml.L^{-1}$
Micronutrients solution*	1.8 $ml.L^{-1}$
Macronutrients solution*	1$ml.L^{-1}$

*The composition of micronutrient and macronutrient solutions was according to Zhender et al. (1980).

Physicochemical analysis

The analyzed variables (or parameters) were collected three times a week. During the reactor operation, pH, alkalinity, dissolved oxygen (DO), COD (chemical oxygen demand) (mg L^{-1}), total Kjeldahl nitrogen (TKN-N) (mg L^{-1}), ammonium nitrogen (NH_4^+-N) (mg L^{-1}), nitrate nitrogen (NO_3^- -N) (mg L^{-1}), nitrite nitrogen (NO_2^- -N) (mg L^{-1}), total suspended solids (TSS), and volatile suspended solids (VSS) (mg L^{-1}) were measured. Alkalinity and pH were measured using a calibrated potentiometer, and alkalinity was determined according to Dilallo and Albertson (1961), modified by Ripley et al. (1986). An Instrutherm MO-900 model oximeter was used to measure DO concentration. All other analyses proceeded according to the Standard Methods for the Examination of Waste and Wastewater (APHA, 2005).

The efficiency of the reactor

The total nitrogen removal rates in each stage were calculated according to Eq. 1. The efficiency of the reactor in nitrification and denitrification was calculated in each stage according to Equations 2 and 3.

TN removal (%) = $\frac{\left(\mathrm{TKN-N+NO_2-N+NO_3^-{-N}}\right)_{\mathrm{in}}-\left(\mathrm{TKN-N+NO_2^-{-N}+NO_3^-{-N}}\right)_{\mathrm{ef}}}{\left(\mathrm{TKN-N+NO_2^-{-N}+NO_3^-{-N}}\right)_{\mathrm{in}}}\times 100$ (Eq. 1)

$\mathrm{E_{nit}}\,(\%) = \frac{\mathrm{TKN-N_{in}-TKN-N_{ef}}}{\mathrm{TKN-N_{in}}}$ (Eq. 2)

$\mathrm{E_{den}}\,(\%) = \frac{\mathrm{TKN-N_{in}-TKN-N_{ef}-NO_3^-{-N_{ef}}}}{\mathrm{TKN-N_{in}-TKN-N_{ef}}}$ (Eq. 3)

In the calculation: TKN-N_{in} is the total Kjeldahl nitrogen influent, NO_2^--N_{in} is the nitrite-N influent, NO_3^--N_{in} is the nitrate-N influent, TKN-N_{ef} is the total Kjeldahl nitrogen effluent, NO_2^--N_{ef} is the nitrite-N effluent and NO_3^--N_{ef} is the nitrate-N effluent.

Due to different COD/N ratios observed in the anoxic chambers; therefore, the comparison among removal efficiencies was made in terms of applied (Equations 4 and 5) and removed rates (Equations 6 and 7):

$\mathrm{Nitrogenloadingrate} = \frac{\mathrm{TKN-N_{in}\times Q}}{\mathrm{V}}$ (Eq. 4)

$\mathrm{Organicloadingrate} = \frac{\mathrm{COD_{in}\times Q}}{\mathrm{V}}$ (Eq. 5)

$\mathrm{TNloadingnitrified} = \frac{\mathrm{(TKN-N_{in}-TKN-N_{ef})\times Q}}{\mathrm{V}}$ (Eq. 6)

$\mathrm{TNloadingdenitrified} = (\mathrm{TNloadingnitrified})\times \mathrm{E_{den}}$ (Eq. 7)

Where COD_{in} is the chemical oxygen demand influent, Q is the flow rate and V is the reactor working volume.

Results And Discussions

Wastewater characteristics

Table 2 shows a general assessment of the results obtained in each experimental stage. The reactor was continuously operated for 252 days with a previous period of nitrifying biomass adaptation of 45 days.

Table 2

– Monitoring of the main SBHBR parameters in chambers 3 (anaerobic effluent) and 5 (anoxic effluent) for TN removal efficiency, nitrification, denitrification, COD loading rate and TN loading rate in stages I, II and III.

	Start-up stage	**Stage I**	**Stage II**	**Stage III**
Influent				
COD (mg $O_2.L^{-1}$)	2583.3 ± 837.3	3012.7 ± 1010.8	2696.8 ± 1192.9	2391.4 ± 592.5
COD loading rate applied (kg $O_2m^{-3}d^{-1}$)	1.29 ± 0.42	1.51 ± 0.51	1.35 ± 0.60	1.20 ± 0.48
TKN-N ($mg.L^{-1}$)	109.05 ± 28.64	163.67 ± 33.17	182.10 ± 53.93	221.87 ± 62.02
pH	7.15 ± 0.35	7.37 ± 0.43	7.11 ± 0.29	6.32 ± 1.74
Anaerobic Effluent Chamber 3				
COD (mg $O_2.L^{-1}$)	249.23 ± 63.62	283.86 ± 87.93	132.35 ± 117.11	51.31 ± 16.26
TN ($mg.L^{-1}$)	87.55 ± 24.96	153.88 ± 42.97	170.75 ± 38.11	151.2 ± 35.49
COD/TN	-	2.07 ± 0.64	0.84 ± 0.51	0.35 ± 0.11
TKN-N ($mg.L^{-1}$)	71.62 ± 23.01	135.2 ± 47.93	175.79 ± 37.88	141.41 ± 34.36
NH_4^+-N ($mg.L^{-1}$)	58.91 ± 26.71	93.18 ± 28.95	124.93 ± 17.90	128.65 ± 28.99
NO_2^--N ($mg.L^{-1}$)	1.44 ± 2.30	2.61 ± 3.36	1.80 ± 3.44	6.26 ± 4.42
NO_3^--N ($mg.L^{-1}$)	19.11 ± 19.49	12.08 ± 18.8	3.12 ± 1.44	2.69 ± 1.31
pH	7.95 ± 0.27	8.01 ± 0.12	7.88 ± 0.19	7.74 ± 0.19
TSS ($mg.L^{-1}$)	25 ± 28	45 ± 11	115 ± 6	35 ± 2
VSS ($mg.L^{-1}$)	9 ± 8	36 ± 16	111 ± 10	2 ± 1
COD removal (%)	91.96 ± 2.93	88.85 ± 6.08	96.13 ± 3.15	97.96 ± 0.73
TN removal – anaerobic conditions (%)	31.6 ± 15.8	23.5 ± 12.3	23.4 ± 16.0	34.8 ± 19.5
TN loading rate applied (kg $N.m^{-3}.d^{-1}$)	0.11 ± 0.05	0.21 ± 0.06	0.25 ± 0.05	0.21 ± 0.05

	Start-up stage	**Stage I**	**Stage II**	**Stage III**
Anoxic Effluent				
COD (mg O_2.L^{-1})	55.83 ± 40.42	113.49 ± 79.81	30.86 ± 43.21	25.25 ± 14.83
TN (mg.L^{-1})	82.7 ± 22.34	100.33 ± 24.38	37.41 ± 19.2	38.06 ± 21.55
NH_4^+-N (mg.L^{-1})	6.27 ± 10.35	23.77 ± 30.21	27.61 ± 29.32	19.84 ± 23.93
NO_2^--N (mg.L^{-1})	0.90 ± 1.60	2.89 ± 3.81	0.18 ± 0.15	0.79 ± 0.61
NO_3^--N (mg.L^{-1})	74.27 ± 19.9	74.83 ± 41.27	13.96 ± 11.76	17.32 ± 9.82
pH	8.17 ± 0.15	8.08 ± 0.18	8.17 ± 0.20	7.63 ± 0.44
TSS (mg.L^{-1})	83 ± 22	34 ± 1	242 ± 37	104 ± 11
VSS (mg.L^{-1})	40 ± 13	26 ± 1	214 ± 54	72 ± 9
Efficiency of nitrification (%)	96.0 ± 4,84	83,0 ± 20,9	85,5 ± 14,4	85,9 ± 17,4
Efficiency of denitrification (%)	-	51.1 ± 29.3	90.7 ± 7.5	85.2 ± 9.05
TN removal (%)	-	43.0 ± 17.5	76.4 ± 12.6	82.3 ± 11.4
TN removal (%) – anoxic condition	27.06 ± 15.89	37.42 ± 16.57	77.39 ± 10.47	74.37 ± 15.0
TN loading removed (kg N.m^{-3}.d^{-1})	0.05 ± 0.04	0.09 ± 0.05	0.19 ± 0.04	0.16 ± 0.05
COD removal (%) – anoxic condition	74.81 ± 9.27	62.14 ± 22.82	81.75 ± 17.17	52.17 ± 21.59
COD removal (%) – the reactor	97.70 ± 1.51	95.97 ± 3.21	99.14 ± 1.15	99.04 ± 0.55
COD loading rate removed (kg $O_2m^{-3}d^{-1}$)	1.23 ± 0.38	1.36 ± 0.52	1.28 ± 0.54	1.18 ± 0.47

COD removal and biomass concentration

The structured-bed hybrid baffled reactor (SBHBR) performed high organic matter removal efficiencies in all the applied experimental conditions. The results in Fig. 2 show that the anaerobic condition in SBHBR played an effective role in the total COD removal, which ranged from 72 to 99% during the operational period. Furthermore, varying the influent COD concentration did not negatively affect COD removal efficiency, which could be associated with the ability of SBHBR to support changes in wastewater characteristics, such as the influent COD concentration.

(Fig. 2 inserted here)

This study observed the highest volumetric COD load rate and the highest removal efficiency at the beginning of stage I with 2.12 kg O_2 m^{-3} d^{-1} and 99,87%, respectively, after 47 days of the total operation. Giordani et al. (2021) operated an anaerobic hybrid baffled reactor treating simulated dairy wastewater and achieved an average removal of COD of 91% with an OLR of 1.0 kgCOD/m³. Santos et al. (2021) also obtained 90% COD removal efficiency at OLR of 1.0 kgCOD/m³ in a hybrid anaerobic biofilm baffled reactor. Jurgensen et al. (2018) studied dairy wastewater using a continuous tank reactor and anaerobic baffled reactor in series and obtained 82% COD removal at OLR between 1.25–4.5 kgCOD/m³.

Overall, these cases support the view that the improved organic matter removal in the SBHBR was possible due to the implementation of intermittent aeration that afforded chambers 4 and 5 to have high mixed liquor. Thus, the intermittent aeration allowed anaerobic and anoxic conditions in the reactor. Then, the remaining effluent COD from the anaerobic chambers 1 to 3 was not only oxidized by aerobic heterotrophic microorganisms but also used as a carbon source by denitrifying bacteria. Thereby, it saved aeration costs. Despite intermittent aeration, the attached and suspended microorganism growth in chambers 4 and 5 may have played an important role in supporting high COD removal efficiencies (HAMODA et al., 2012).

The influent COD concentrations in chambers 4 and 5 differed depending on the operational stage due to the previous anaerobic processes (chambers 1 to 3), which presented different COD removal efficiencies in each stage. The highest value for the initial COD concentration was observed during stage I. Thus, the average efficiency for COD removal in the anoxic zone during stage I was 62.14 ± 22.82%. The same parameter for stage II achieved better performance with a higher COD removal efficiency of 81.75 ± 17.17% when the COD concentration in chamber 4 observed was 132.35 ± 117.11 mg L^{-1}.

The high treatment performance could be associated with the high biomass concentration, contributing to high removals of organic matter. The reactor efficiently retained biomass in the system as shown in Fig. 3. Stage II demonstrated the maximum effluent concentration of VSS 214 mg.L^{-1}, which agrees with the major COD removals discussed earlier. Leyva-Díaz et al. (2016) operated a hybrid moving bed biofilm bioreactor-membrane containing carriers in the anaerobic, anoxic, and aerobic zones (hybrid MBBR-MBR) treating municipal wastewater and obtained an effluent with a high concentration of volatile suspended solids of 3.75 g L^{-1}. Thus, the lower VSS registered in the SBHBR effluent and the high efficiency of retention VSS in the reactor can be addressed to the polyurethane foam, facilitating greater biomass retention inside the SBHBR. The heterogeneous composition of the retained biomass in the SBHBR system may have enabled better reactor performance regarding the organic matter and nitrogen removal efficiencies. Furthermore, in the steady state, NT removal had a negligible impact on nitrogen assimilation; thus, it was possible to observe a low sludge yield in the biological process.

(Fig. 3 inserted here)

Nitrogen removal

The following part of this paper describes the total nitrogen removal of SBHBR, as this system enabled advanced nitrogen removal under different environmental conditions. The total nitrogen removal all over the operation of SBHBR in stages I, II, and III were 43.0 ± 17.5%, 76.4 ± 12.6%, and 82.3 ± 11.4%, respectively. The most likely cause for the lower TN removal in stage I was that the denitrifying bacteria was not fully established in chambers 4 and 5. These results are likely related to the high availability of organic matter. Heterotrophs bacteria are essential to provide growth factors for autotrophs (JI et al., 2021). Thus, when there was a decrease in the C/N ratio, an increase in nitrification in the system was observed.

Removal of TN in the anaerobic zone was 23.5 ± 12.3%, 23.3 ± 16.0%, and .34.8 ± 19.5%. The data reported here support the assumption that in chambers 1, 2, and 3, cellular assimilation occurred mainly in the removal of influent TN; once in the anaerobic system, the ammonia assimilation causes the TN removal by the heterotrophic biomass generated (BONASSA et al., 2021). However, the nitrification and denitrification processes established in chambers 4 and 5 contributed more significantly to the TN removal process in the reactor. Figure 4a shows that the average nitrogen removal in chambers 4 and 5 was predominant in the total nitrogen removal in the reactor.

As far as chambers 4 and 5 are concerned, the TN removal in stage I was lower (37.4 ± 16.6%) compared to stages II and III, which showed TN removal of 77.4 ± 10.5% and 74.6 ± 14.7%, respectively. As explained earlier, the denitrifying biomass had insufficient time to fix in the polyurethane foam under intermittent aeration, indicating a high concentration of NO_3^--N as shown in Fig. 4b. The low concentration of NO_2^--N in the bulk liquid can be attributed to high nitrification efficiency. What can be seen in Fig. 4a is the high TN removal at the final of the operation (last 3 points) of stage I. The increase in TN removal was from 24.4% to up 70.2%, indicating that taken 55 days to establish the denitrification of microorganisms in the bioreactor. However, adapting the denitrification process is essential in maintaining high nitrogen removal in the RCH (SILVA et al. 2022).

(Fig. 4 inserted here)

During the start-up stage, chambers 4 and 5 were maintained under non-stoppable aeration for 45 days, which contributed to the oxidization of 99% of NH_4^+-N in the bulk liquid. Figure 5 shows the nitrification and denitrification efficiencies, the concentration of NH_4^+-N influent and effluent, and the pH values. These results demonstrated that simultaneous nitrification and denitrification processes occurred in chambers 4 and 5 under intermittent aeration, once stages I to III removed total nitrogen. The approach used, aerating the bulk liquid with no addition of electron donor and alkalinity source for the denitrification occurrence, was previously used by other researchers (BARANA et al., 2013, WENDLING et al., 2022). Therefore, it was observed that the employment of intermittent aeration did not profoundly affect the denitrifying bacteria (Fig. 5a).

Furthermore, the electron donor source from the anaerobic chambers was sufficient to promote total nitrogen removal in the SBHBR. In addition, alkalinity production in anaerobic conditions was observed in all stages. It was also noted that the pH kept adequate for TN removal (Fig. 5c), allowing the simultaneous processes of nitrification and denitrification in a single environment (in the chambers 4 and 5) under intermittent aeration Therefore, analyses performed for nitrogen removal were restricted to these chambers.

(Fig. 5 inserted here)

Another significant aspect of TN removal is that the highest efficiency was 94%, achieved during stage II under COD/NT ratio of 0.84, resulting in the highest TN loading removed (0.25 ± 0.05 kgN $m^{-3}d^{-1}$). In stage II, NH_4^+-N, NO_2^--N, and NO_3^--N concentrations in the effluent were, on average, 24.6 ± 29.3 mg L^{-1}, 0.18 ± 0.15 mg L^{-1}, and 13.9 ± 11.7 mg L^{-1}, respectively.

The mean values observed at steady state (last five samples) for the total nitrogen removal from each stage were statistically analyzed by ANOVA, and the means were compared by the Tukey test ($p = 0.05$). It was observed that TN removal efficiency in stage I indicated a statistical difference compared to the other two stages. Stage II and III were not statistically different.

The nitrification efficiency values for the start-up stage and stages I, II, and III were 96.0 ± 4.9%, 83.0 ± 20.9%, 85.5 ± 14.4%, and 85.9 ± 17.4%. Nitrification efficiency of stages I to III, established and measured in chambers 4 and 5, did not report statistical difference when considering values from the period of operation of the reactor when considering steady-state (the last 5 points collected in each stage). ANOVA statistically correlated the means of the evaluated results. Leyva-Díaz et al. (2013) observed that immobilized biomass had enhanced the development of slow-growth microorganisms, such as nitrifying bacteria. These microorganisms remain longer in the system due to the structure of foam, which can give rise to their attached growth by potentially forming biofilms (MOURA et al., 2012). This information could support the results of the nitrification efficiencies that were achieved when operating SBHBR under intermittent aeration.

Denitrification efficiency was 51.1 ± 29.3%, 89.8 ± 7.2%, and 85.19 ± 9.05% for stages I, II, and III, respectively. Overall, the instability of denitrifying biomass in stage I contributed to the decline of denitrification. Thus, during this stage, the denitrification efficiency increased from 24.5–97.2% in 71 days of operation (13 taken samples), leading to a denitrification efficiency average value of 51.1 ± 29.3%. During the first 40 days of operation, the average denitrification efficiency was 23.8 ± 8.3%. From that mark to the last day of stage I operation, the average denitrification efficiency increased to 74.5 ± 16.4%, denoting that the acclimatization of denitrification occurred during this period. In this context, the statistical analysis was performed when the reactor presented steady-state characteristics. Comparative statistical analysis of denitrification efficiencies based on Kruskal-Wallis (significance level of $p \leq 0,05$) showed no significant differences between all stages.

The relationship between denitrification and organic matter consumed can be determined by the stoichiometric demand of 4.2 gCOD/g N. As mentioned above, the effluent average concentration values for soluble organic matter in stages I, II, and III were 113.5 ± 79.8 mg.L^{-1}, 30.9 ± 43.2 mg.L^{-1}, and 25.2 ±

14.8 mg.L^{-1}. Thus, the theoretical organic matter consumed in stages I, II, and III by the denitrification process were 225, 560, and 475 mg COD.L^{-1}, respectively. However, the differences in COD concentrations between the anaerobic and anoxic zone were 170.4, 101.5, and 26.1 mg COD.L^{-1}, respectively. Thereby, the consumed COD was lower than the denitrification process required.

Thus far, the evidence supports the idea that hybrid systems can provide suitable conditions for endogenous denitrification bacteria, where intracellular carbon sources act as the electron donor to drive nitrite or nitrate reduction (LO et al. 2010; WINKLER et al. 2011). The assumption that simultaneous biological processes occurred during SBHBR operation was also supported by the capacity of aerobic and anoxic zones to be formed in the superficial of the reactor and inner niches. Recent research has revealed that alternating anaerobic/anoxic conditions have an advantageous effect on the specific growth of endogenous denitrifying bacteria (CHANG et al.; 2021; FENG et al., 2022; ZHANG et al., 2022). In addition, in all conditions, the mean concentration of NO_2^--N was beneath 2.9 mg NO_2^--N L^{-1}. Huang et al. (2022) stated that a low concentration of NO_2^--N could lead to simultaneous biological processes, such as carbon oxidation, nitrification, anammox, and denitrification.

Considering the removal of 1g of N without assimilation, 1.14g of COD is needed to reduce nitrate (NO_2^-) to nitrous oxide (N_2O) (Saggar et al., 2013). Traditional nitrogen removal, considering biological synthesis via nitrate, requires 4.0g COD/gN, while modified nitrogen removal via nitrite requires 2.4 g COD/gN (AKUNNA et al., 1992). One way of evaluating the performance of denitrifying bacteria in SBHBR was to investigate the TN removal from organic matter and the theoretical COD/N ratio based on stoichiometric calculations for the theoretical removal of total nitrogen via nitrous oxide, nitrite, and nitrate.

Table 3 depicts the TN removal balance carried out in the three operational conditions. It was observed in stage III that the real TN removal efficiencies via nitrous oxide, nitrite, and nitrate are noticeably higher than the theoretically calculated TN removal efficiencies. At this stage, the real TN removal efficiency was 82.8%, and the theoretical TN removal efficiency via nitrous oxide, nitrite, and nitrate was 10.0%, 6.6%, and 4.0%, respectively. By contrast, the results of stages I and II, as shown in Table 3, indicate that the real TN removal efficiencies were not different from the theoretically calculated TN removal efficiencies.

Table 3
– Average concentrations of COD removal, TN removed, COD $_{removed}$/TN$_{removed}$, real TN removal, TN removal via nitrous oxide, TN removal via nitrite, and TN removal via nitrate.

Stages	**COD removal (kg N.m^{-3}.d^{-1})**	**TN removed (kg N.m^{-3}.d^{-1})**	**COD$_{removed}$/TN$_{removed}$ ratio**	**Real TN removal (%)**	**TN removal via nitrous oxide (%)**	**TN removal via nitrite (%)**	**TN removal via nitrate (%)**
I	0.278	0.150	2.33	56.3	77.0	51.2	30.7
II	0.247	0.186	1.35	82.5	97.5	63.8	38.4
III	0.025	0.159	0.157	82.8	10.0	6.6	4.0

Undoubtedly, the operational heterogeneity of the SBHBR made it possible to remove nitrogen from dairy wastewater with a low COD/TN ratio. Intermittent aeration conditions caused anaerobic and anoxic zones in the same chambers, consolidating different functional capacities of the bacteria. Almeida et al. (2018) operated a structured-bed reactor under low aeration with a reduced recirculation ratio in post-treating animal feed wastewater with a COD/TN ratio of 0.28. They achieved a TN removal of 48%. Meng et al. (2015) studied an upflow microaerobic sludge reactor (UMSR) when treating manure-free piggery wastewater source with a COD/TN ratio of about 0.84 and achieved TN removal of 87.2% when the UMSR reached a steady state. Therefore, there was evidence that under lower COD/TN ratios (0.26–2.9) (GAO et al., 2013, ZHANG et al., 2020; SANTOS et al., 2021) and intermittent aeration (WANG et al., 2022; WEN et al., 2022; MIAO et al., 2022), the nitrogen removal process may be associated with anaerobic ammonium oxidation observed in anammox processes as the main mechanism for TN removal.

The concentration of DO in chambers 4 and 5 bulk liquid was 3.0 to 0.1 mgO$_2$ L^{-1} along the stages. According to Rodriguez et al.(2011), the DO concentration presents a linear correlation with the concentration of nitrifying bacteria. Nonetheless, even when DO concentration was lower, nitrifiers probably maintained high nitrification efficiency due to biofilm formation. The low COD/TN ratios may have inhibited the heterotrophic bacteria decreasing competition with nitrification bacteria for available OD in the bulked liquid (MOURA et al., 2018), thus increasing the nitrification process in chambers 4 and 5. Thus, this study suggests that the restriction of organic matter (low C/N ratio) combined with the stratification of the biofilm layers resulted in the selection of nitrifying microorganisms instead of heterotrophic bacteria.

The high TN removal efficiencies obtained in the steady states of all stages suggested that autotrophic denitrification may have positively influenced the performance of the SBHBR. As a result, the wide range of COD observed in the anaerobic/anoxic zone of the SBHBR allowed an ideal development for anammox bacteria. In addition, the intermittent aeration proposed for chambers 4 and 5 increased microorganisms with slow growth rates, such as anammox bacteria.

Conclusions

A new reactor concept was designed to combine the benefits of baffled and biofilm-structured reactors. The advantages of using polyurethane foam strips under intermittent aeration indicate an improvement in the removal of organic matter and total nitrogen and a reduction in the concentration of suspended solids. The high volatile solids retention in the SBHBR system and the formation of anoxic and aerobic niches in the reactor were achieved thanks to the polyurethane foam used as the reactor bed. The reactor operational strategy enabled an efficient TN maximum of 91.9% and COD removal maximum of 99.8% in stage III at COD/N ratios of 0.35 and under a low and intermittent aeration regime after some period of biomass acclimatization (stage I). The results of this study indicate that the TN removal efficiency increased from 43.0 ± 17.5% to 82.3 ± 11.4% when the influent COD/TN ratio decreased from 2.1 ± 0.6 to 0.35 ± 0.1. Moreover, the intermittent aeration and the biofilm formation may have contributed to the occurrence of anoxic zones in the reactor, which allowed simultaneous nitrification and denitrification with no need for extra carbon source addition or recirculation approaches. These findings may have played an important role in scaling up the SBHBR reactor as the need for a post-treatment clarification could be suppressed, or its volume could be scaled down, also saving aeration costs and simplifying the setup with no recirculation.

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' contributions

Danilo S. G. Lucio: Methodology, Formal analysis, Data Curation, Writing – Original Data, Writing – Review & Editing

Maria Eduarda S Dias: Conceptualization, Writing – Original Data, Writing – Review & Editing.

Rogers Ribeiro: Conceptualization, Writing – Review & Editing, Supervision

Giovana Tommao: Conceptualization, Writing – Review & Editing, Resources, Supervision, Project Administration, Funding Acquisition.

ETHICAL APROVAL

Not Applicable

CONSENT TO PARTICIPATE

All authors consented to participate in this manuscript.

CONSENT TO PUBLISH

All authors have consented to publish this manuscript.

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Figures

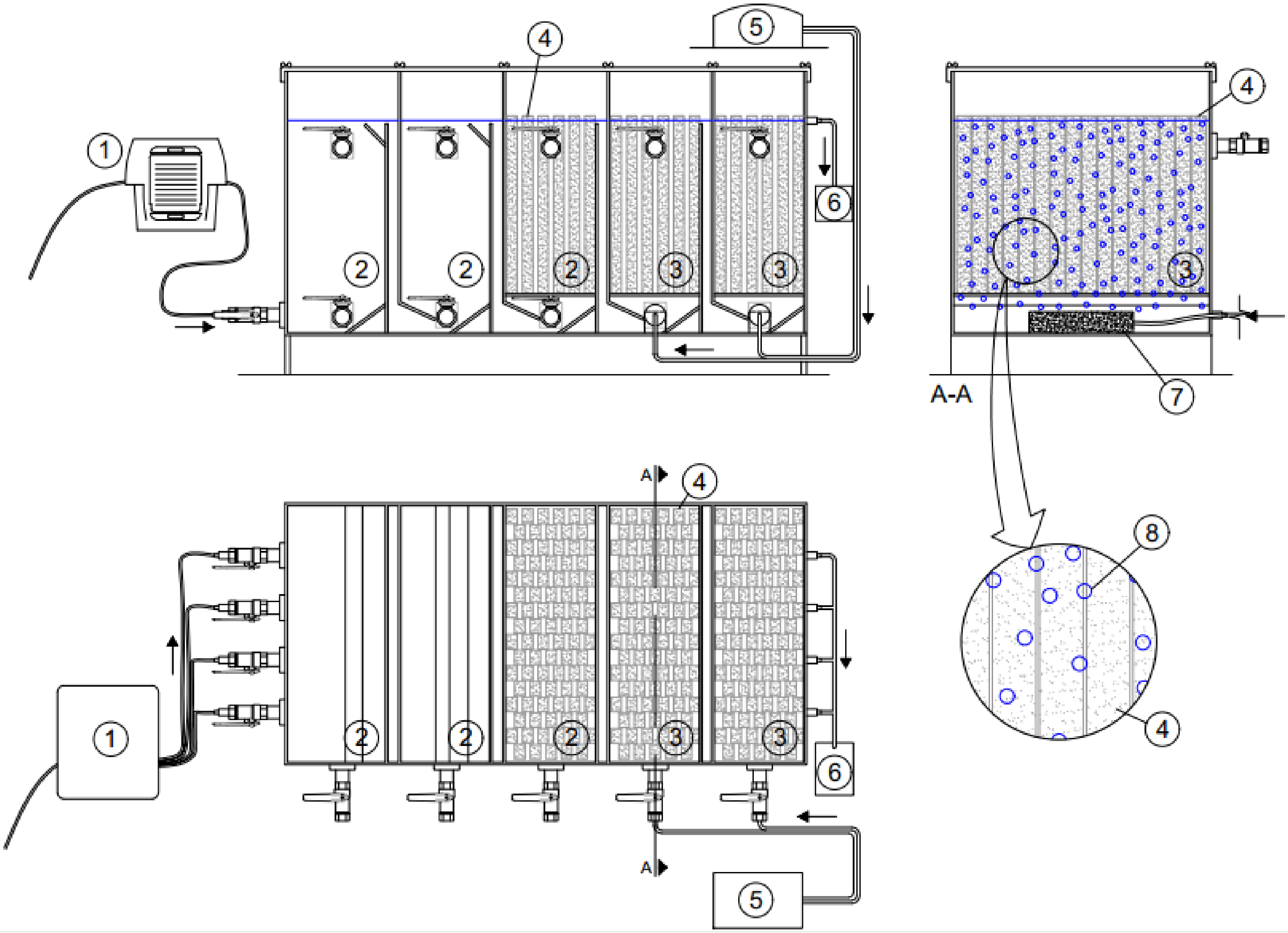

Figure 1

Structured-bed Hybrid Baffled Reactor (SBHBR). 1- Inflow pump. 2- anaerobic zone. 3- anoxic zone. 4- polyurethane foam. 5- air compressor. 6-outflow. 7- porous stone stick. 8- air bubbles.

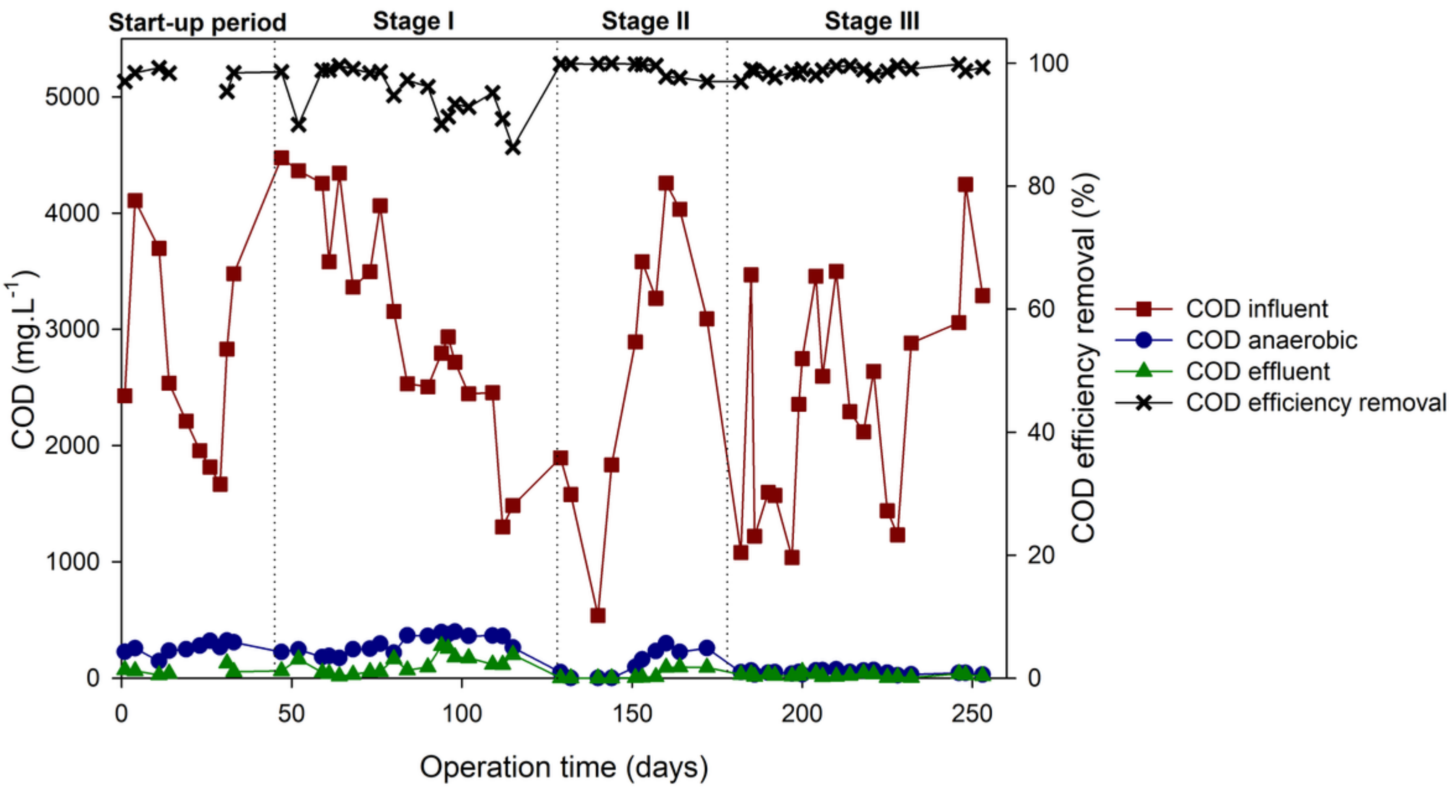

Figure 2

Profiles of influent, influent anaerobic, effluent COD, and total removal efficiencies of COD in SBHBR.

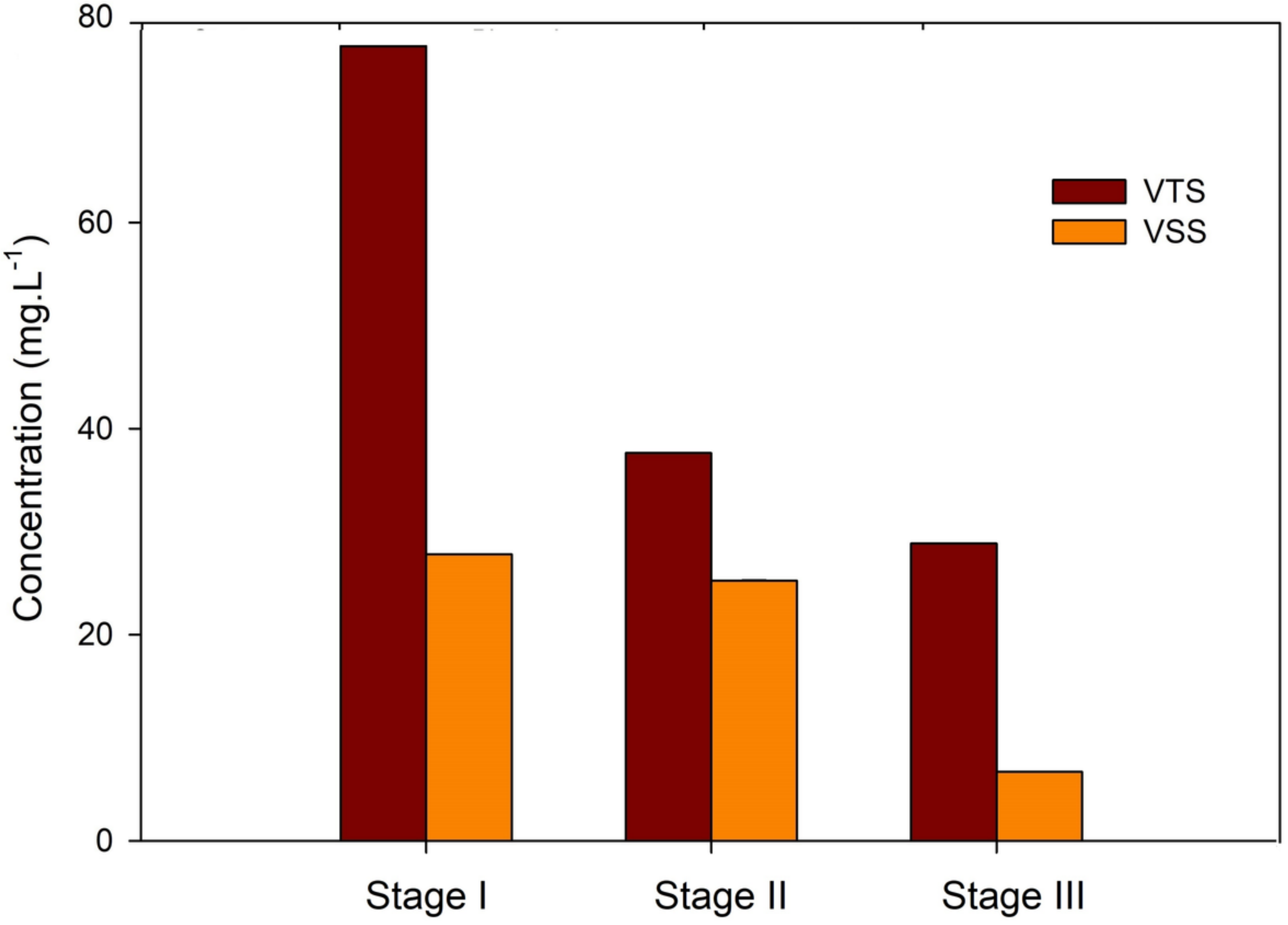

Figure 3

Average concentration of volatile total solids (VTS) and volatile suspended solids (VSS) in the SBHBR effluent.

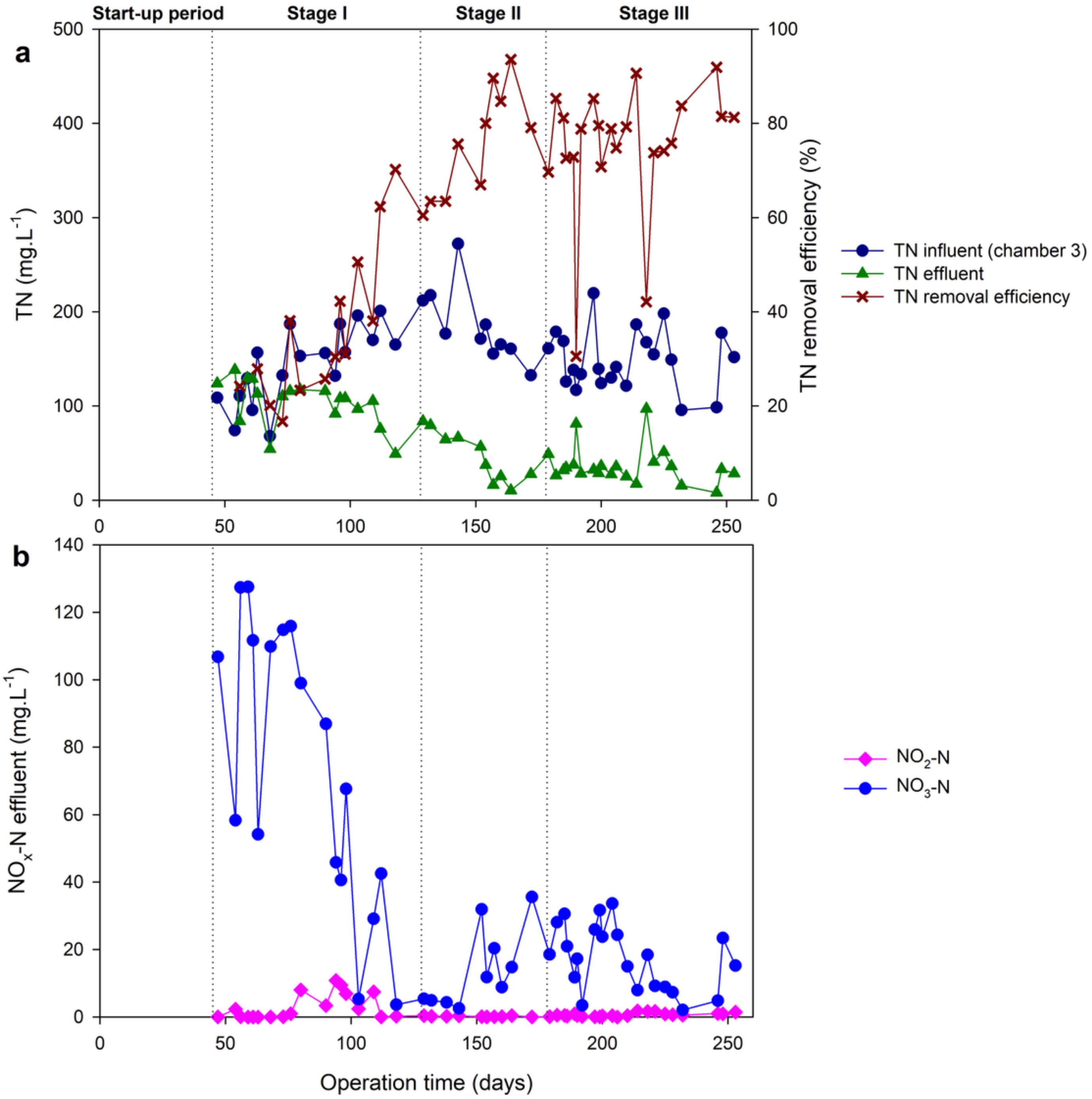

Figure 4

Performance of the SBHBR in total nitrogen, nitrite, and nitrate concentrations.

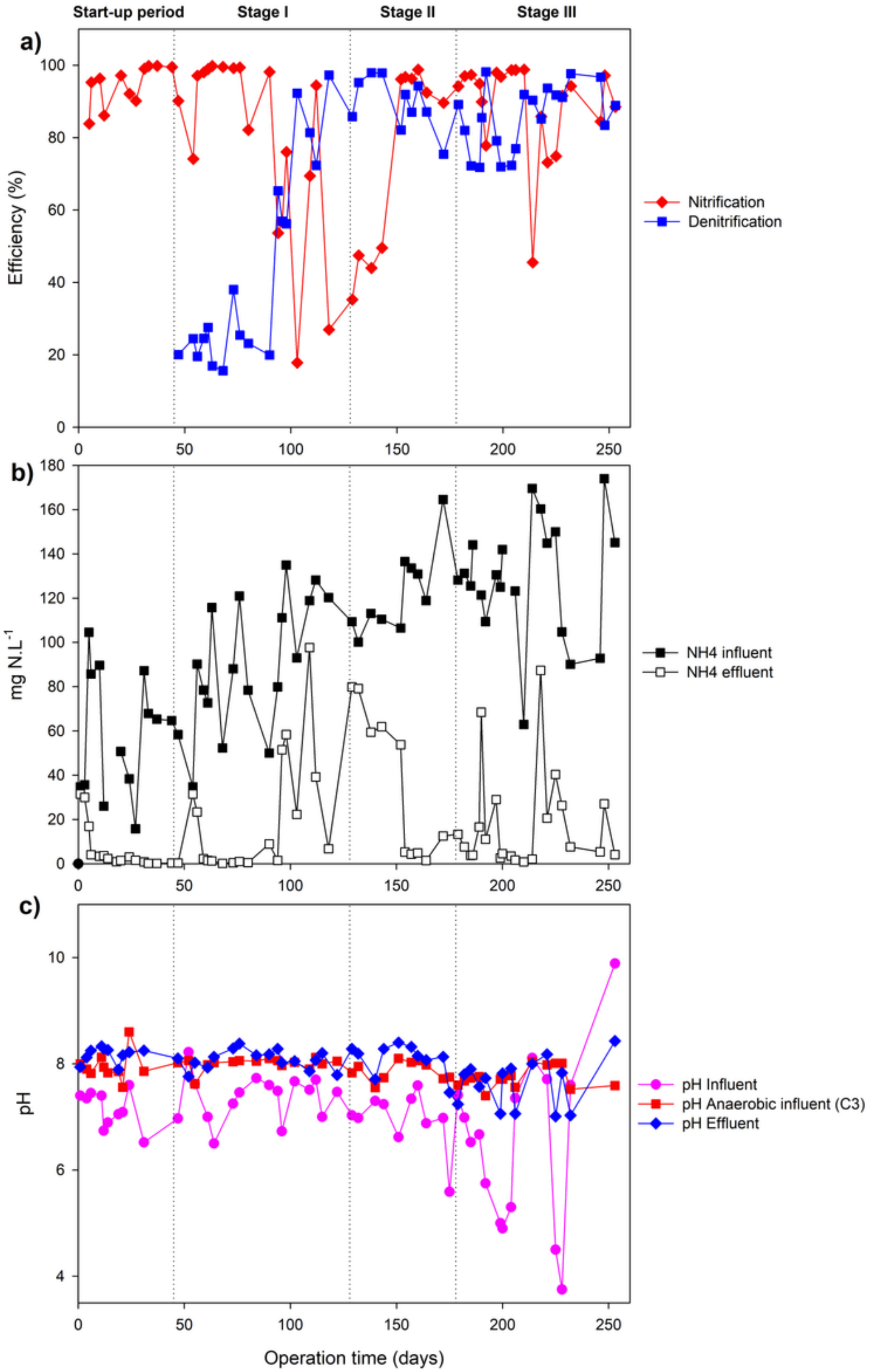

Figure 5

Performance of the SBHBR in nitrification, denitrification, NH4+ influent, NH4+ effluent, and pH