Investigation of the effect of nitrification inhibition on the performance and effluent quality of aerobic sequential batch reactors

İrem Uslu · Hüseyin Yazıcı

Abstract The use of nitrification inhibition as a concentrating step for ammonium (NH_4^+), for the purpose of increasing the potential for simultaneous recovery of phosphate (PO_4^{3-}) and NH_4^+ from effluent streams of an aerobic sequencing batch reactor (SBR) system, has never been investigated in the literature. Therefore, the present study aimed to determine the effect of the inhibition of nitrification on both the reactor performance and effluent quality in a laboratory scale aerobic SBR system. In order to compare the observed results, a separate reactor, where the inhibition was not applied, was operated as a control reactor (CR) under the identical operational conditions used for the inhibitory reactor (IR). Experimental results for the reactor performance showed that effluents with low total suspended solids (<50 mg/L) and chemical oxygen demand concentrations ($>90\%$ of removal efficiency based on the influent concentration of 500 mg/L) were achieved for both SBRs by obtaining an activated sludge with a sludge volume index <60 mL/g after the acclimation period. In the same period, the effluent PO_4^{3-}, NH_4^+, and nitrate (NO_3^-) concentrations were found to be 17.0 ± 4.0, 1.26 ± 0.84, and 21.5 ± 39 mg/L for the CR and 10.0 ± 4.4, 3.9 ± 2.4, and 9.2 ± 1.5 mg/L for the IR, respectively. During this period, 94% of the removed NH_4^+ ($NH_4^+_{rem.}$) was converted to NO_3^- in the CR, indicating almost complete nitrification occurred in the reactor. However, only 47% of the $NH_4^+_{rem.}$ was converted to NO_3^- in the IR as a result of the inhibition of nitrification, meaning a partial inhibition (53%) occurred due to the inhibition treatment. These results clearly demonstrated that the inhibition of nitrification allowed the effluent NH_4^+ concentrations to increase by suppressing the formation of NO_3^- ions. Based on the results, it can be concluded that inhibition of nitrification in an aerobic SBR system creates a potential for conserving the effluent NH_4^+ concentration and increasing consecutive recovery of PO_4^{3-} together with NH_4^+ from the effluent discharges.

Keywords Sequencing batch reactor · Activated sludge · Nitrification · Inhibition · Reactor performance · Effluent quality

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Introduction

Primary nutrients such as nitrogen (N) and phosphorus (P) are the most essential elements that are crucial to all known forms of life (Ahmed et al., 2019;

Jiaying et al., 2022; Jupp et al., 2021). Despite their positive effects in water habitats such as supporting fish and shellfish production, these nutrients cause several negative impacts due to chemical fertilizers or discharged wastewaters (Kim & Chae, 2016; Wurtsbaugh et al., 2019). In terms of the discharged wastewaters, the excess volume of stormwater and wastewater received in wastewater treatment plants (WWTPs) is released into aquatic environments as untreated sewer overflow due to the limited capacity of WWTPs (Sojobi & Zayed, 2022). Increases in such anthropogenic inputs of N and P to aquatic environments trigger rapid growth of algae and higher forms of plant life, causing increased consumption of oxygen and often leading to declining water quality along the freshwater to coastal areas (Malone & Newton, 2020; Manav, 2006). As a result, rapid spikes in nutritive substances in aquatic ecosystems cause various environmental problems including red tides, eutrophication, and other environmental damage (Alvarez et al., 2017; Chen et al., 2022; Kim & Chae, 2016; Wang et al., 2018). Eutrophication creates multiple problems such as releasing greenhouse gases, limitation of cultural and social values of waters, creation of hypoxic "dead zones" that reduce fish and shellfish production, and formation of harmful algal blooms that create taste and odor problems and threaten the aquatic food supplies and safety of drinking water (Wurtsbaugh et al., 2019). Thus, N- and/or P-rich untreated wastewaters pose a significant risk to human health and the environment (Chen et al., 2022). Therefore, N and P removal from wastewaters by effective treatment technologies is crucial to meet the strict discharge standards and limits the environmental problems caused by the discharge of nutrients to aquatic environments (Emparan et al., 2019; Rout et al., 2021).

To overcome the abovementioned environmental problems in receiving water bodies, water quality legislations have increased the standards for removal of nutrients (Pastor et al., 2008). In the last two decades, scientists around the world have made many improvements to conventional processes and have developed many new technologies that exceed the current treatment level with a low operating cost potential (Oleszkiewicz et al., 2015). Before disposing the effluents to the environment, the removal of carbonaceous organic matter, total suspended solids (TSS), chemical pollutants, excess nutrients, and pathogens have been the principal objective of

wastewater treatment (Chrispim et al., 2019). Transforming the WWTPs, which are traditionally energy intensive and expensive, with resource recovery mechanisms, is an emerging objective due to environmental and economic benefits, such as reducing nutrient discharges to the environment and reducing a considerable amount of energy required for clean drinking water consumption (Ahmed et al., 2015; Chrispim et al., 2019; Mehta et al., 2015; Oyegoke et al., 2012; Rao et al., 2017; Sojobi, 2016).

In a conventional biological treatment plant, P recovery can occur using several technologies designed for its recovery from untreated wastewater, plant effluents, liquid phases, sewage sludge, and sewage sludge ash (Egle et al., 2016; Urdalen, 2013). Considering that high P concentrations are generated after enhanced biological P removal (EBPR) and sludge digestion, often causing problems in the sludge stream, P recovery from the side streams of WWTPs (the reject water created from the separation of liquids and solids, typically involving dewatering and thickening processes) was assigned a higher priority than the recovery from the effluent stream. On the other hand, most of the contaminants existing in wastewater are removed in the effluent stream, making it a valuable nutrient resource, especially for agricultural applications in terms of its potential use as irrigation water. However, only a small proportion of the P compared to the initial concentration in wastewater can be recovered, since it is eliminated from the effluent stream in all larger WWTPs. To increase the potential of P recovery from the effluent, it should not be eliminated from the effluent stream (Sartorius et al., 2012).

N removal techniques transit ammonium (NH_4^+) ions existing in the wastewaters to N gas ($N_{2(g)}$) that is ultimately lost to the atmosphere. Through biological N removal (nitrification–denitrification), there is also an increased potential for NO_x emissions due to the reactive intermediates in the process, the impact of which can reach up to 80% of the total operational carbon footprint of the treatment plant (Desloover et al., 2012; Shenk, 2021). This energy intensive step is important in returning the water quality to an appropriate level and also represents a lost opportunity for resource recovery by allowing the removed N to escape to the atmosphere (Shenk, 2021). Therefore, examining more sustainable pathways for N, such as the direct recovery of N from wastewater and its reuse, becomes a relevant issue. Unfortunately,

studies showing the recovery of N from wastewater that was combined with its reuse at full scale are limited (van der Hoek et al., 2018). Precipitation of NH_4-N as struvite has been applied in practice, but the focus of this process was the recovery of P (van der Hoek et al., 2017). By taking the increasing anthropogenic sources of N into consideration, several studies have attempted to shift the focus towards the recovery of N in addition to P, to not only prevent eutrophication, but to also minimize the energy consumption and save natural resources (Beckinghausen et al., 2020). Considering the ongoing growing tendency of local crop production and the treatment of large volumes of wastewater in WWTPs, the recovery of N and P as struvite has become especially attractive for urban areas (Rufí-Salís et al., 2020). Several studies have proven that fertilizer quality of struvite is comparable to that of conventional fertilizers (Latifian et al., 2012; Liu et al., 2011; Peeva et al., 2021; Siciliano, 2016; Szymanska et al., 2020). Moreover, the struvite recovered from dewatered filtrate of anaerobic sludge digestion and commercially produced in Japan was sold to fertilizer companies (Ueno & Fujii, 2001).

Currently, several methods have been investigated for their effectiveness in nutrient recovery, including traditional methods such as chemical precipitation and adsorption, and more advanced approaches such as bioelectrochemical systems and osmotic membrane bioreactors (Ye et al., 2020). Among these, the recovery of nutrients from wastewater by struvite precipitation has gained increasing interest in recent years (Siciliano et al., 2020). This approach was also supported during a study by Saliu and Oladoja (2021), which outlined primary research studies examining the recovery of P and N by analyzing several recovery methods from different nutrient-rich wastewaters originating from agricultural, industrial, and municipal sources. Most of the methods reported in the studies were relying on struvite precipitation.

Hallas et al. (2019) reported P recovery from aerobically digested municipal wastewater. The study noted that struvite formation is favored in the large WWTPs when expensive technologies, such as anaerobic processing, are being used because small WWTPs are relying on aerobic treatment, which is often lacking in capital, economic scale, operational staffing, and physical operational area. In addition, converting smaller WWTPs to anaerobic treatment

(more conducive to struvite formation) is typically cost prohibitive. Therefore, it is not surprising that large WWTPs have been targeted for developing struvite recovery strategies, leaving the smaller WWTPs behind. For such small aerobic WWTPs, concerns over how to improve the quality of effluents in terms of N and P parameters come to the forefront.

Inlet wastewater streams are characterized by high volumes but low concentrations and are difficult to recover nutrients from without a concentrating step (Beckinghausen et al., 2020). This aspect might also be valid for effluent streams of WWTPs. By considering that biological removal of phosphate (PO_4^{3-}) requires an anaerobic selector (Oehmen et al., 2007), which is not involved in aerobic treatment, and NH_4^+ ions are oxidized to NO_x ions through nitrification in aerobic treatment (Rodríguez-Gómez et al., 2021), a concentrating step may inevitably be required for NH_4^+ ions rather than PO_4^{3-} ions in aerobic WWTPs, if the nutrient recovery from effluent streams through struvite precipitation is targeted. This means that the nitrification needs to be suppressed or, if possible, completely prevented in the aerobic treatment in order to maximize NH_4^+ concentrations in the effluents for its recovery. However, due to the requirement of a concentrating step for NH_4^+ ions, the recovery of NH_4^+ together with PO_4^{3-} from effluent streams of aerobic treatment systems has not yet been examined until now, to the current knowledge of the authors.

Ammonia oxidation is the first rate-limiting step of nitrification (Lehtovirta-Morley et al., 2013). Nitrification inhibitors can inhibit the activities of the responsible ammonia-oxidizing microorganisms and related enzymes, effectively delaying the oxidation of NH_4^+ to NO_3^-, thereby alleviating NO_3^- accumulation and leaching loss and maintaining a high NH_4^+ content (Wang et al., 2021). In agricultural fields, these inhibitors have frequently been found to prolong the retention time of NH_4^+ in the soil, thereby increasing the N use efficiency of fertilizers, and reducing the impact of serious environmental problems, including NO_3^- pollution, air quality degradation, and greenhouse gas emissions (Guo et al., 2021; Nardi et al., 2020; Ruser & Schulz, 2015; Wang et al., 2021). In environmental studies, the inhibition of nitrification by adding inhibitory agents (e.g. N-allylthiourea, ATU) into the inlet streams has also been applied in EBPR studies using sequencing batch reactors (SBRs) (Flowers et al., 2009; Ge et al., 2013; Pacheco, 2018; Rubio-Rincon

et al., 2017; Yazıcı & Kılıç, 2016), in order to prevent the deterioration of EBPR resulting from the presence of nitrite $(NO_2^-)/(NO_3^-)$ or oxygen in the anaerobic zone (Rey-Martinez et al., 2021). A variety of compounds have been used to inhibit nitrifying prokaryotes, including aliphatic and aromatic n-alkynes, plant-derived molecules, and statins, and many of these are used as selective inhibitors in laboratory cultures, soil microcosms, or slurries. Unfortunately, these are not suitable due to their rapid degradation in soil or application in a gaseous state (Papadopoulou et al., 2020). Among the most frequently used commercial nitrification inhibitors, ATU was used as an inhibitory agent in the present study because it is a known specific inhibitor for NH_4^+ oxidizers, suppressing the oxygen uptake (Lin et al., 2021; Liu et al., 2018), and has been frequently used in nitrification research (Lehtovirta-Morley et al., 2013; Tatari et al., 2017).

The effect of several operational parameters on the treatment performance of aerobic SBRs has been investigated comprehensively in the literature. On the other hand, recovery of N and/or P from different waste streams using various techniques has also received much attention in recent years. In this study, an attempt was made to investigate whether inhibiting the nitrification step could be applied as a concentrating step for NH_4^+ ions and used as a strategy to suppress the formation of NO_2^- or NO_3^- ions, thus conserving the NH_4^+ concentration in the effluent stream of a laboratory-scale aerobic SBR system. To the authors' best knowledge, in order to maximize the effluent NH_4^+ concentration for a potential nutrient recovery process, the effect of inhibiting the nitrification step on the reactor performance and effluent quality of a SBR system has not been investigated in the literature until now. Therefore, the present study aimed to determine the effect of the inhibition of nitrification on both the reactor performance and effluent quality in laboratory scale aerobic SBR systems. The main objectives of the study were to (i) determine the reactor performance according to chemical oxygen demand (COD) removal efficiency, sludge settleability, and effluent TSS concentration under certain operational conditions, (ii) to determine the effluent quality based on the NO_2^-, NO_3^-, NH_4^+, and PO_4^{3-} concentration in the effluent, and (iii) to compare the observed results for the the inhibitory reactor (IR) with those of the control reactor (CR), in which the inhibition was not applied under the identical operational conditions.

Materials and methods

Reactor operation

In this study, the operation of aerobic SBR systems was designed (i) to remove COD and TSS at a high removal efficiency, and (ii) to increase the effluent NH_4^+ concentration by suppressing the formation of NO_3^- ions using a nitrification inhibitor. For this purpose, synthetic wastewater solutions representing the characteristics of inlet wastewater of a typical domestic WWTP (Fig. 1) were treated in the SBRs under certain operational conditions. The SBRs with a working volume of 5 L were operated simultaneously under the identical operational conditions, except for the inhibition of nitrification applied in the IR. In the CR, the nitrification was not inhibited during the entire operational period. The SBRs were operated for 32 days, including an acclimation period of 15 days. The inoculum was supplied from the oxic zone of an activated sludge (AS) unit at the Lara Advanced WWTP (Antalya, Turkey).

The cycle times, synthetic wastewater solution volume, and calculated values for hydraulic retention time (HRT) and organic loading rate (OLR) are given in Table 1. In order to improve the reactor performance, the acclimation period was divided into three operational phases of 5 days each. For this purpose, each of the phases (phases 1 to 3) consisted of different operational strategies, such as reducing the HRT by decreasing the cycle times, shortening the time dedicated for settling of the AS in the SBRs, and increasing the OLR by increasing the volume of the feeding solution (volume exchange ratio, VER). A cycle of 12 h per day was applied as a longer cycle (famine phase) during the entire operational period. Starting from the 6th day of the acclimation period, one of the 12-h cycles was applied as two 6-h cycles per day for the next 5 days (phase 2), and from the 11th day as three 4-h cycles per day (feast phase) for the next 5 days (phase 3).

During the entire reactor operation, the filling phase was completed in the first 10 min of the cycle while the reaction mixture was aerated but not mixed externally. Sludge settling was performed for 19 min on the 1st day, and the duration was decreased to 10 min at the end of the 10th day by decreasing it for 1 min daily during phases 1 and 2. In phases 3 and 4, the settling time was applied as 10 min until the
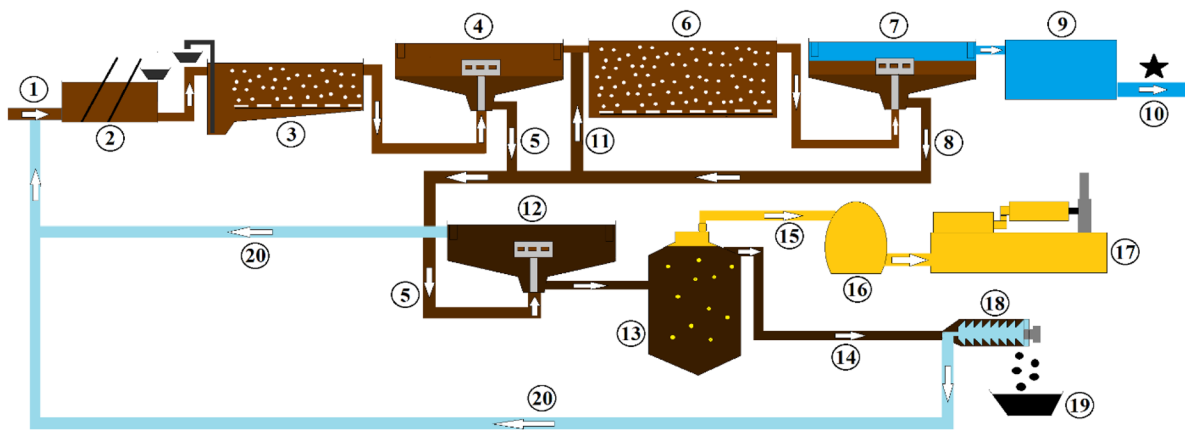
Fig. 1 Schematic diagram of a typical domestic WWTP (1: Inlet wastewater; 2: Coarse and fine screens; 3: Aerated grit chamber; 4: Primary settling tank; 5: Settled sludge; 6: Activated sludge unit; 7: Secondary settling tank; 8: Waste activated sludge; 9: Disinfection and final treatment unit; 10: Effluent; 11: Return activated sludge; 12: Gravity thickener; 13: Anaerobic digester; 14: Digested sludge; 15: Biogas; 16: Biogas storage tank; 17: Cogeneration system; 18: Decanter centrifuge; 19: Dewatered sludge; 20: Reject water. The location focused in the present study)

reactor operation was completed. Since the settling time was decreased by 1 min each day, the duration of the aeration cycle was correspondingly increased from 691 to 695 min between the days 1 and 5 (phase 1). Between the days 6 and 10 (phase 2), the time for aeration was increased from 696 to 700 min in the 12-h cycle and from 336 to 340 min in the 6-h cycle. For the remaining operational period (phases 3 and 4), it was set to 700 min in the 12-h cycle and 220 min for the 4-h cycle (Table 1).

The volume of the synthetic wastewater solution was 2.5 L per cycle (50% of VER) in the first 5 days of the acclimation period (phase 1), and then it was increased to 3 L per cycle (60% of VER) for the following operation phases (Table 1). At the beginning of each cycle, a fresh synthetic wastewater solution was fed to the reactors as the same volume of the effluent discharged at the end of each cycle. For filling of the synthetic wastewater and discharging of the effluent, two peristaltic pumps, which were operated

Table 1 Cycle times and operational conditions of the SBRs

	Acclimation period			After acclimation period
	0–5 days **(phase 1)**	**6–10 days** **(phase 2)**	**11–15 days** **(phase 3)**	**(phase 4)**
Cycle time (hour) per day	12/12	12/6/6	12/4/4/4	12/4/4/4
Phases of a cycle (time, minute)				
Filling and aeration	691–695	696–700/336–340	700/220	700/220
Settling	19 to 15	14 to 10	10	10
Effluent withdrawal	10	10	10	10
HRT (hour)	24	20[a]	20[a]	20[a]
		10[b]	6.66[c]	6.66[c]
Mean HRT (hour)	24	15	13.33	13.33
Volume of feeding solution (liter per cycle)	2.5	3	3	3
OLR (kg COD/m³.day)	0.5	0.9	1.2	1.2

[a]HRT value corresponding to 12-h cycle between the days 6 and 10

[b]HRT value corresponding to 6-h cycle between days 6 and 10

[c]HRT value corresponding to 4-h cycle between days 11 and 32

by both clockwise and anticlockwise directions, were used for each SBR. During the aerobic phases, mixing was carried out from the bottom side of the reactor at a constant speed of 900 rpm through a magnetic stirrer (Fig. 2). The SBRs were operated without adjusting the sludge retention time (SRT) to a constant value. In both SBRs, a certain volume of mixed liquor was wasted from the SBRs at the end of each cycle just before the settling period (a total volume of 200 mL daily). The corresponding SRT value was calculated based on the measured concentration of the mixed liquor suspended solids (MLSS) and effluent TSS concentration.

Aeration was done through a rod-shaped flexible aquarium diffuser that circled around the bottom side of the reactor. An aquarium aerator, which allowed the air flow to be adjusted to the desired value, was used as the air supply. The flow rate of the air was controlled with an air flow meter by supplying the air at a flow rate of 1 L/min so that the dissolved oxygen concentration measured in the mixed liquor was kept around 5 mg/L. When the air flow rate decreased below 1 L/min, or an obvious biofilm formed on the wall of the reactor vessel, the diffuser and the interior parts of the reactor were cleaned manually. Before and after cleaning of the reactor, the dissolved oxygen concentration in the mixed liquor was measured using a portable oxygen meter (Milwaukee MW600). The pH in the SBRs was not controlled during the entire operation period, but instead was measured at the beginning and end of each cycle using a portable pH meter (Milwaukee Mi150). Whenever the pH value was measured below 7.0, after the synthetic wastewater solution was fed into the reactor, it was adjusted to a desired value of 7.0–7.2 with 1 N NaOH

solution. The temperature of the mixed liquor was not controlled, but the actual temperature was routinely monitored together with pH measurements.

Synthetic wastewater

Synthetic wastewater solution was prepared as a four-cycle stock solution as required. The solution was fed to the reactor as three separate solutions named Solution A, Solution B, and NH_4Cl solution. At the beginning of each cycle, 0.75 L of Solution A, 2 L of Solution B, and 0.25 L of NH_4Cl solution was fed to the reactors sequentially to form the final feeding solution. Since the total volume of the feeding solution was 2.5 L during the first 5 days of the acclimation period, Solution A, Solution B, and NH_4Cl solutions were prepared by proportioning their respective volumes into 3 L of total volume. Solution B contained 4 mL of a trace element solution per liter. The trace element solution was prepared as 1 L stock solution, as described in Lu et al. (2006), and was added to Solution B as required after the stock Solution B was prepared. In the IR, where nitrification was inhibited, ATU ($C_4H_8N_2S$) was included in Solution B at a concentration of 0.07 mM (Tatari et al., 2017).

Composition of the synthetic wastewater solutions added to both reactors is outlined in Table 2. The required amounts of each compound in the feeding solution were calculated according to the following conditions that were applied to ensure the desired concentration balance between the compounds was achieved (Yazıcı & Kılıç, 2016): (i) C/N/P: 100/10/5, (ii) $\Sigma M/\Sigma D$: 1.5, indicating the ratio of the total monovalent cation concentration (ΣM, meq/L) resulting from NH_4^+ and K^+ ions to the total divalent cation
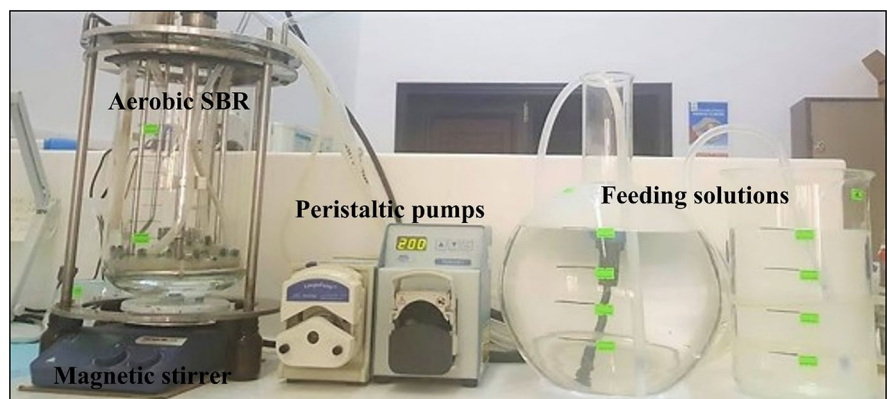
Fig. 2 Photo of the reactor setup

Table 2 Composition of synthetic wastewater solution

	Concentration		Concentration
<u>**NH$_4$Cl solution (0.25 L/ cycle)**</u> NH$_4$Cl (nitrogen source)	71.4 mg/L (N: 18.7 mg/L) (NH$_4$$^+$: 24.1 mg/L) (NH$_4$$^+$: 1.33 meq/L)	<u>**Solution B (2 L/cycle)**</u> MgSO$_4$.7H$_2$O (magnesium source) CaCl$_2$.2H$_2$O (calcium source) KCl (potassium source) ATU (inhibitor) *Trace element solution	89.1 mg/L (Mg^{2+}: 0.724 meq/L) 53.1 mg/L (Ca^{2+}: 0.724 meq/L) 40.0 mg/L (K$^+$: 0.536 meq/L) 70 µM (per liter) 4 mL/L
<u>**Solution A (0.75 L/ cycle)**</u> C$_6$H$_{12}$O$_6$.H$_2$O (carbon source) KH$_2$PO$_4$ (phosphorus source)	516 mg/L (COD: 500 mg/L) (C: 187 mg/L) 41.0 mg/L (P: 9.35 mg/L) (PO$_4$: 28.6 mg/L) (K$^+$: 0.301 meq/L)		

*Trace element solution was added on the following trace elements (mg/L): CuSO$_4$.5H$_2$O: 30, FeCl$_3$.6H$_2$O: 1500, ZnSO$_4$.7H$_2$O: 120, KI: 180, H$_3$BO$_3$: 150, MnCl$_2$.2H$_2$O: 120, Na$_2$MoO$_4$.2H$_2$O: 60, CoCl$_2$.6H$_2$O: 150, EDTA: 10.000

concentration (ΣD, meq/L) resulting from Mg^{2+} and Ca^{2+} ions, and (iii) Mg^{2+}/Ca^{2+}: 1/1 (Higgins & Novak, 1997), indicating the ratio of the Mg^{2+} concentration (in meq/L) to the Ca^{2+} concentration (in meq/L).

Sampling and analytical procedures

Analyses were carried out every 2 days during all reactor operational phases, including the acclimation period. Samples were taken at the end of both 12-h cycle and 4- or 6-h cycles. Samples of 50 mL were taken at the end of the cycle while the reactor was mixing. Depending on the analytical procedures applied, analyses were carried out either on mixed liquor samples or on filtrate samples obtained by filtration of the mixed liquor. TSS analyses were performed only for effluent samples of 50 mL. Samples were analyzed according to the relevant analysis procedure immediately after they were taken. MLSS, mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) analyses were performed on mixed liquor samples, while COD, NH$_4$$^+$, NO$_3$$^-$, NO$_2$$^-$, and PO$_4$$^{3-}$ analyses were performed on filtrate samples. For SVI analysis, 1 L of mixed liquor samples were taken at the end of the sampling cycle while the reactor was mixing. After the settling procedure was completed in the Imhoff cone, the supernatant was drained, and the remaining sludge was added back into the reactor before the next cycle started, thus attempting to prevent possible biomass losses due to the sampling for SVI analysis.

TSS-MLSS and MLVSS analyses were performed according to the APHA 2540D and 2540E method, respectively (APHA, 1999). For the 2540D method, samples were filtered through Millipore AP40 filter paper, and drying was carried out in an oven (Nüve FN500). For the 2540E method, filter papers were ignited in a furnace (Protherm PLF 110/6). Tare of filter papers and the weights after drying and ignition procedures were determined by a microbalance (Radwag AS220/C12). SVI analyses were performed according to the APHA 2710D method (APHA, 1999). For this method, the volume of settleable solids was determined according to the volumetric test procedure (2540F.3a). Determination of COD concentrations was carried out through calibration curves, prepared by measuring absorbances and plotting the values against the reference solutions at 600 nm (for the solutions having COD concentration greater than 90 mg/L) and 420 nm (for the solutions having COD concentration less than 90 mg/L) wavelengths in a spectrophotometer (WTW, SpektroFlex 6100 VIS) according to the closed reflux colorimetric method as described in the APHA 5220D method (APHA, 1999). In this method, the digestion procedure was carried out on a thermoreactor (WTW, CR2200). Concentration of PO$_4$$^{3-}$, NH$_4$$^+$, NO$_2$$^-$ and NO$_3$$^-$ were measured colorimetrically in the spectrophotometer by using Merck Spektroquant test kits.

Data evaluation

The collected data were analyzed using GrapdPad Prism version 9.1.2 (GraphPad Software). Means were compared using the Student's *T* test between

the SBRs, with the level of significant difference defined as $P < 0.05$. The correlation structure between the reactor performance parameters and effluent quality parameters was determined using Pearson's correlation (r) coefficient with statistical significance set at $P < 0.05$. The r values for the IR and CR were represented in a single matrix and interpreted using the following classifications (Mukaka, 2012): very strong positive (negative) correlation: 0.90 to 1.00 (-0.90 to -1.00), strong positive (negative) correlation: 0.70 to 0.90 (-0.70 to -0.90), moderate positive (negative) correlation: 0.50 to 0.70 (-0.50 to -0.70), low positive (negative) correlation: 0.30 to 0.50 (-0.30 to -0.50), negligible positive (negative) correlation: 0.00 to 0.30 (0.00 to -0.30).

Results and discussions

Evaluation of the reactor performance

The main design criteria for SBR systems are to obtain a granular sludge with higher settling velocity, by washing-out floccular sludges from the system, which are less dense and have a lower settling velocity than granular sludge. The time allocated for settling the sludge is a key design parameter to ensure granular sludge formation by washing-out the floccular biomass, and also that the settling time is optimally short enough to provide selectivity for biomass particles that can ultimately settle in a short time (Beun et al., 1999). In the current study, the performance of the reactors was mainly evaluated according to sludge settleability, effluent TSS concentration, and COD removal efficiency. MLSS, SRT, MLVSS, and food to mass (F/M) parameters were also evaluated to guide the interpretation of the reactor performance.

MLSS and SVI profile of the SBRs

The data was analyzed to determine the MLSS concentrations and SVI values, and the results are presented in Fig. 3. According to the results obtained for the IR, the MLSS concentration in the first 5 days of the reactor operation (phase 1) decreased to 1073 mg/L from an initial value of 2584 mg/L

(Fig. 3a). This decrease was the result of biomass loss during the discharge of effluent at the end of the cycle depending on the decrease in the settling performance of the sludge during the first 5 days (SVI increased from 190 to 280 mL/g). At the end of the following 10-day period (on the 15th day), the SVI value decreased to 108 mL/g and the MLSS concentration increased to 3148 mg/L.

The results obtained for the CR indicated that the change in MLSS concentration and the SVI during the acclimation period showed a similar trend as compared to the IR (Fig. 3b). According to the results, the MLSS concentration decreased to 1160 mg/L and the SVI value increased to 232 mL/g at the end of the first 5 days (phase 1). As a result of the changes in the operational conditions (as applied in the IR, gradually decreasing the HRT value and daily shortening of the settling time) applied on the 6th day, the SVI value decreased to 99 mL/g, and the MLSS concentration increased to 3211 mg/L on the 15th day.

In both reactors, further increase in MLSS concentrations and further decrease in SVI values were observed during the 17 days of operation after the acclimation period (phase 4). At the end of the reactor operation, the MLSS concentration increased to 5600 mg/L and SVI value decreased to 37.6 mL/g for the IR. In the CR, these parameters were recorded as 4512 mg/L and 47.3 mL/g for the MLSS and SVI, respectively.

As shown in Fig. 3, the changes in the MLSS concentration and the SVI exhibited similar trends during each of the phases in both reactors. A similar finding was also observed by Solomon et al. (2019). The result of the T test analysis revealed that the variations for both of the MLSS and SVI parameters in each phase did not show significant differences ($P > 0.05$) between the SBRs (Table 3). According to the results of Pearson correlation analysis (Table 4), there was a very strong significant negative correlation between MLSS and SVI for the IR ($r = -0.92$; $P < 0.01$) and a strong significant negative correlation for the CR ($r = -0.78$; $P < 0.05$) during the acclimation period. These results suggested that the fluctuating settling performance of the sludge during the acclimation period (especially in phases 1 and 2) was the driving force, causing the change in the MLSS concentration in both reactors. In phase 4, MLSS showed a moderate significant negative correlation with SVI ($r = -0.65$, $P < 0.01$)
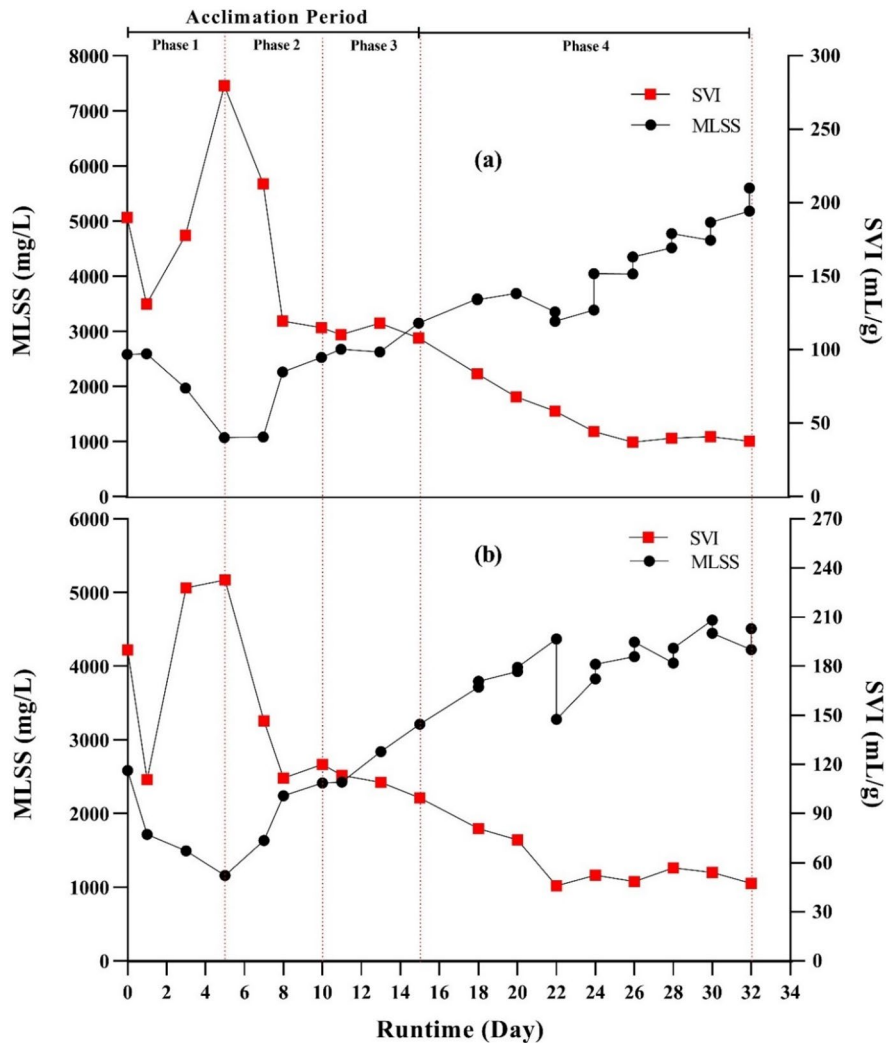
for the IR. However, interestingly, no significant correlation ($r = -0.36$) was recorded for the CR although the variation in MLSS and SVI showed a similar trend as compared to the IR (Fig. 3). Very strong significant positive correlations between the MLSS and MLVSS parameters ($r = 0.97$ to 0.99; $P < 0.01$) were also observed for both reactors during the entire operational period.

*Effluent TSS concentration and SRT profiles
of the SBRs*

Effluent TSS concentrations and the corresponding SRT values are presented in Fig. 4. According to the results obtained for the IR (Fig. 4a), the effluent TSS concentration fluctuated during the acclimation period

depending on the change in the SVI value and the increase in OLR. Although the MLSS concentration increased gradually between days 16 and 32 (phase 4), there was no remarkable biomass loss during the discharge of the effluent in the IR due to the increase in the sludge settling performance. Thus, the effluent TSS concentration remained below 75 mg/L (mean value 48.6 ± 15.2 mg/L) during phase 4. The highest effluent TSS concentration measured for the IR in phase 4 was 74 mg/L on the 22nd day. It can be seen that the SRT value decreased when the TSS concentration increased, because the SRT value is inversely correlated with effluent TSS concentration. This correlation was confirmed by the results of the correlation analysis (Table 4). According to the results, a strong significant negative correlation was observed

Table 3 Results of the *T* test analysis for the reactor performance parameters

Phase	Reactor performance parameters		Significance (P value)
	IR	CR	
SVI (mL/g)			
1	194.6 ± 62.0	190.3 ± 56.4	Non-significant ($P > 0.05$)
2	149.1 ± 55.3	126.1 ± 18.3	Non-significant ($P > 0.05$)
3	112.1 ± 5.2	107.3 ± 6.9	Non-significant ($P > 0.05$)
4	51.1 ± 17.0	57.4 ± 12.9	Non-significant ($P > 0.05$)
TSS (mg/L)			
1	152.7 ± 254.1	14.0 ± 17.4	Non-significant ($P > 0.05$)
2	16.6 ± 5.0	12.0 ± 4.0	Non-significant ($P > 0.05$)
3	208.7 ± 145.3	42.6 ± 13.3	Non-significant ($P > 0.05$)
4	48.6 ± 15.2	33.1 ± 19.2	Significant ($P = 0.017$)
COD removal (%)			
1	92.3 ± 1.4	95.5 ± 2.4	Non-significant ($P > 0.05$)
2	91.6 ± 0.6	95.5 ± 1.0	Significant ($P = 0.005$)
3	95.0 ± 1.7	98.9 ± 0.6	Significant ($P = 0.021$)
4	92.5 ± 3.5	98.2 ± 1.1	Significant ($P < 0.0001$)

between the SRT and TSS parameters for the IR during the acclimation period ($r = -0.88$; $P < 0.01$) and in phase 4 ($r = -0.72$; $P < 0.01$). Considering the MLSS concentration increased from 3588 to 5600 mg/L, it should be noteworthy to mention that the SRT value did not change remarkably after the acclimation period. Even though the biomass concentration increased, and a certain volume of the biomass was routinely wasted from the reactor on a daily basis, the reactor operation was accomplished under a SRT condition ranging between 5.7 and 19.7 days (mean value 14.1 ± 3.7 days) in phase 4.

The experimental results obtained for the CR revealed that trends for the effluent TSS concentration and SRT value during the acclimation period were quite similar with that of the IR (Fig. 4b). Analysis between the TSS and SRT parameters showed that

a very strong ($r = -0.95$; $P < 0.01$) and moderate ($r = -0.59$; $P < 0.05$) significant negative correlation was observed for the CR during the acclimation period and in phase 4, respectively. The result of the *T* test revealed that the variations for both of the TSS and SRT parameters in each phase (phases 1 to 3) and the variations for the SRT parameter in phase 4 were not significantly different ($P > 0.05$) between the SBRs. However, there was a slight difference ($P = 0.017$) between the SBRs in terms of the TSS parameter in phase 4, which could be attributed to the difference between the mean values. During phase 4, the effluent TSS concentration remained below 70 mg/L (mean value 33.1 ± 19.2 mg/L), while the SRT value ranged between 7.5 and 23.0 (mean value 16.0 ± 4.5 days).

Jadhao and Dawanse (2013) reported that membrane bioreactor (MBR) systems, which can be operated under longer SRT conditions compared to conventional biological treatment systems, allow for more increase in treatment efficiency due to the high biomass concentration, and that some MBR systems can be operated under infinite SRT conditions due to this advantage. In our preliminary study (Yazıcı & Uslu, 2020), an aerobic SBR system was operated under an infinite SRT condition, meaning that no AS was wasted during the entire operation period of 52 days. In that study, the results showed that the SRT value ranged between 15 and 53 days during the acclimation period of 15 days and it increased up to 173 days after the following operation period of 21 days, with the increasing MLSS concentration and sludge settling performance up to 3148 mg/L and 35 mL/g, respectively. However, due to such an increase in the SRT, there occurred a deflocculation in the sludge and, consequently, deterioration in the treatment performance of the SBR (e.g., decrease in MLSS, sludge settling performance, and COD removal and an increase in effluent TSS concentration) in the following operation period of 17 days. Tsilogeorgis et al. (2008) studied the effect of different operational conditions on the treatment of landfill leachate in a membrane SBR system operated for 4 months under the infinite SRT condition. The results showed that the MLSS concentration increased from 7000 to 15,300 mg/L, but the COD removal efficiency could only be achieved in the range of 40–60% during the study period. The apparent failure of the reactor to achieve higher COD removal efficiency

Table 4 Pearson correlation coefficients (r) between the reactor performance parameters and effluent quality parameters for the IR and CR

		MLSS	MLVSS	SVI	TSS	SRT	F/M	COD	PO_4^{3-}	NH_4^+	NO_3^-
		Acclimation period (phases 1–2-3)									
		IR									
CR	Parameter	MLSS	MLVSS	SVI	TSS	SRT	F/M	COD	PO_4^{3-}	NH_4^+	NO_3^-
	MLSS	1	**0.99**[**]	**−0.92**[**]	0.19	−0.40	−0.53	**−0.68**[*]	0.37	0.52	0.05
	MLVSS	**0.99**[**]	1	**−0.93**[**]	0.20	−0.41	−0.52	−0.65	0.38	0.46	0.12
	SVI	**−0.78**[*]	**−0.77**[*]	1	−0.17	0.42	0.36	0.50	−0.51	−0.34	−0.20
	TSS	**0.67**[*]	0.66	−0.19	1	**−0.88**[**]	−0.22	−0.06	−0.15	0.09	−0.07
	SRT	−0.63	−0.63	0.18	**−0.95**[**]	1	0.00	0.36	−0.21	−0.03	0.01
	F/M	0.04	0.08	−0.07	0.10	−0.33	1	0.09	0.48	−0.63	0.11
	COD	**−0.72**[*]	**−0.68**[*]	0.64	−0.63	0.61	−0.04	1	−0.42	−0.56	0.32
	PO_4^{3-}	−0.18	−0.15	0.65	0.37	−0.40	0.24	0.36	1	−0.10	0.06
	NH_4^+	0.63	0.61	−0.59	0.55	−0.51	−0.01	−0.61	−0.37	1	−0.57
	NO_3^-	0.42	0.41	0.09	0.54	−0.59	−0.08	−0.21	0.58	0.25	1
		After acclimation period (phase 4)									
		IR									
CR	Parameter	MLSS	MLVSS	SVI	TSS	SRT	F/M	COD	PO_4^{3-}	NH_4^+	NO_3^-
	MLSS	1	**0.99**[**]	**−0.65**[**]	0.06	0.36	**−0.99**[**]	**0.82**[**]	−0.44	−0.03	−0.29
	MLVSS	**0.97**[**]	1	**−0.66**[**]	0.08	0.35	**−0.99**[**]	**0.84**[**]	−0.48	−0.07	−0.28
	SVI	−0.36	−0.45	1	−0.53	0.04	0.62	−0.48	0.34	0.35	0.11
	TSS	−0.10	−0.05	0.09	1	**−0.72**[**]	−0.05	0.07	−0.51	−0.11	0.45
	SRT	0.09	0.06	−0.11	**−0.59**[*]	1	−0.37	0.28	0.12	−0.29	−0.46
	F/M	**−0.97**[**]	**−0.99**[**]	0.40	0.09	−0.06	1	−0.41	0.46	0.07	0.30
	COD	0.30	0.20	−0.15	0.12	−0.02	−0.23	1	−0.38	−0.13	−0.26
	PO_4^{3-}	−0.12	−0.07	0.33	−0.29	0.06	0.06	−0.23	1	0.49	−0.07
	NH_4^+	0.34	0.33	−0.11	−0.04	0.13	−0.31	−0.11	−0.06	1	0.18
	NO_3^-	−0.26	−0.20	−0.04	−0.08	0.40	0.20	−0.13	0.05	**−0.53**[*]	1

[*]Significance at 0.05 level and

[**]significance at 0.01 level

was associated with the high sludge age as well as the degradability of some compounds such as humic and fulvic acid present in the leachate. On the other hand, Hajiabadi et al. (2009), who carried out the study using four identical SBRs operated under SRT conditions of 5–10-15–20 days, found out that the COD removal efficiencies, MLSS concentrations, and sludge settling performances of the reactors increased and the effluent TSS concentrations decreased as the SRT value increased. As suggested by Tsilogeorgis et al. (2008), an appropriate amount of sludge should be wasted from the reactor, and its operation should be carried out under an appropriate SRT value in order to control the activity of the microbial community. Morgenroth et al. (1997) found out that the SRT value changed as a result of the settling velocity of

the sludge particles. In that study, it was concluded that the slow-settling flocs were washed-out from the SBR reactor during discharging of the effluent, and, therefore, the SRT value was equal to the HRT value in such a case. On the other hand, the fast-settling flocs remained in the reactor at longer SRT values, creating a selective pressure that resulted in enrichment of these flocs. The results of the current study obtained for the MLSS, SVI, TSS, and SRT parameters (Figs. 3 and 4) were in good accordance with the abovementioned literature findings.

MLVSS and F/M profiles of the SBRs

The change in the MLVSS concentration and the F/M ratio for the IR and CR is presented in Fig. 5. In the

Fig. 4 Variations of effluent TSS concentration and SRT in the reactors. (**a**) IR, (**b**) CR
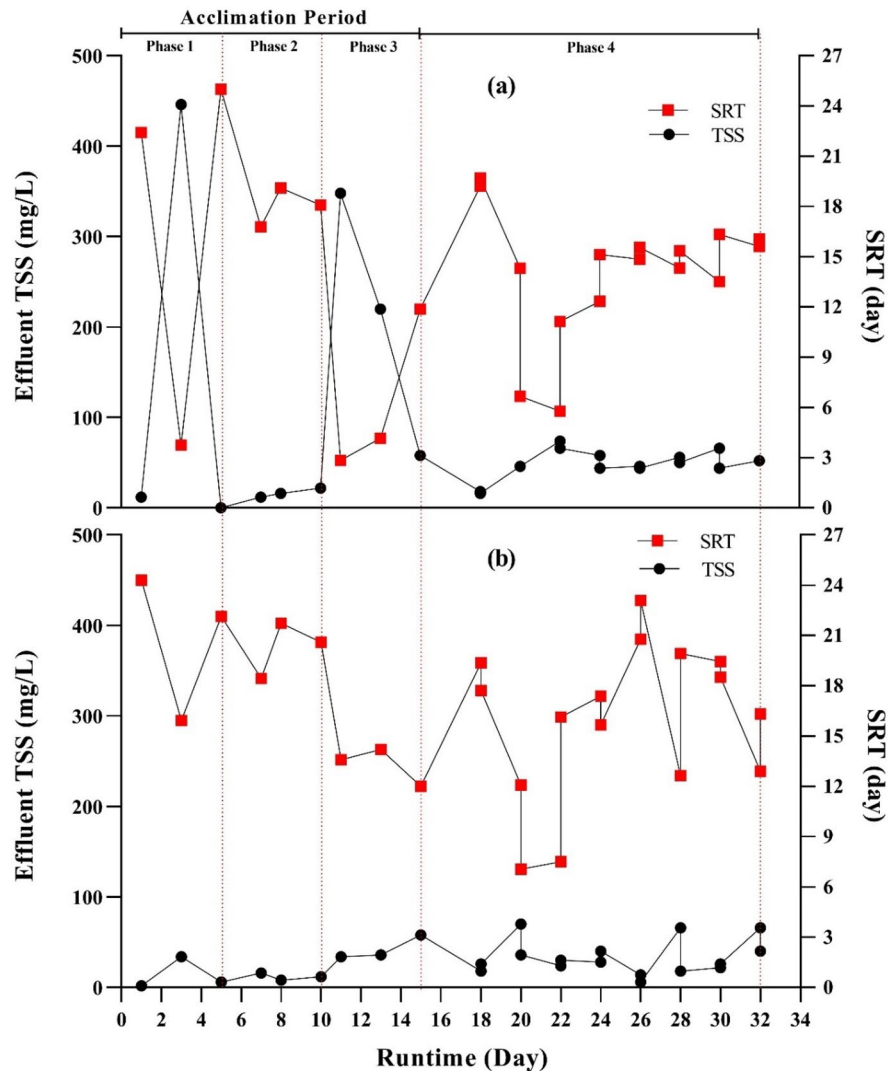

IR, the change in the MLVSS concentration followed a trend similar to that of change in the MLSS concentration (Fig. 5a) due to the very strong significant positive correlation ($r=0.99$; $P<0.01$) between MLSS and MLVSS (Table 4). The MLVSS/MLSS ratio (data not shown) over the operational period was calculated to be 84.0% on average. On the other hand, the F/M ratio fluctuated due to the gradual increase in the OLR value and the change in the MLSS concentration during the acclimation period, resulting in a non-significant correlation between the MLVSS and F/M parameters (Table 4). The mean value of the F/M ratios was calculated as 0.35 ± 0.15, 0.59 ± 0.28, 0.50 ± 0.0, and 0.35 ± 0.0 kg COD/kg MLVSS.day for phases 1, 2, 3, and 4, respectively. These results implied that

the F/M value of the IR gradually decreased with the increasing OLR. Correspondingly, a very strong significant negative correlation ($r=-0.99$; $P<0.01$) was recorded between the MLVSS and F/M parameters in phase 4.

The change in the MLVSS concentration and F/M ratio in the CR and the correlations between these parameters showed a trend similar to that of the IR during the entire operational period (Fig. 5b and Table 4). The significant correlations between the MLVSS and F/M parameters observed for the IR were also recorded for the CR during the entire operational period. The result of the *T* test revealed that the variations for both of the MLVSS and F/M parameters in each phase did not show a significant difference

**Fig. 5** Variations of MLVSS and F/M in the reactors. (**a**) IR, (**b**) CR
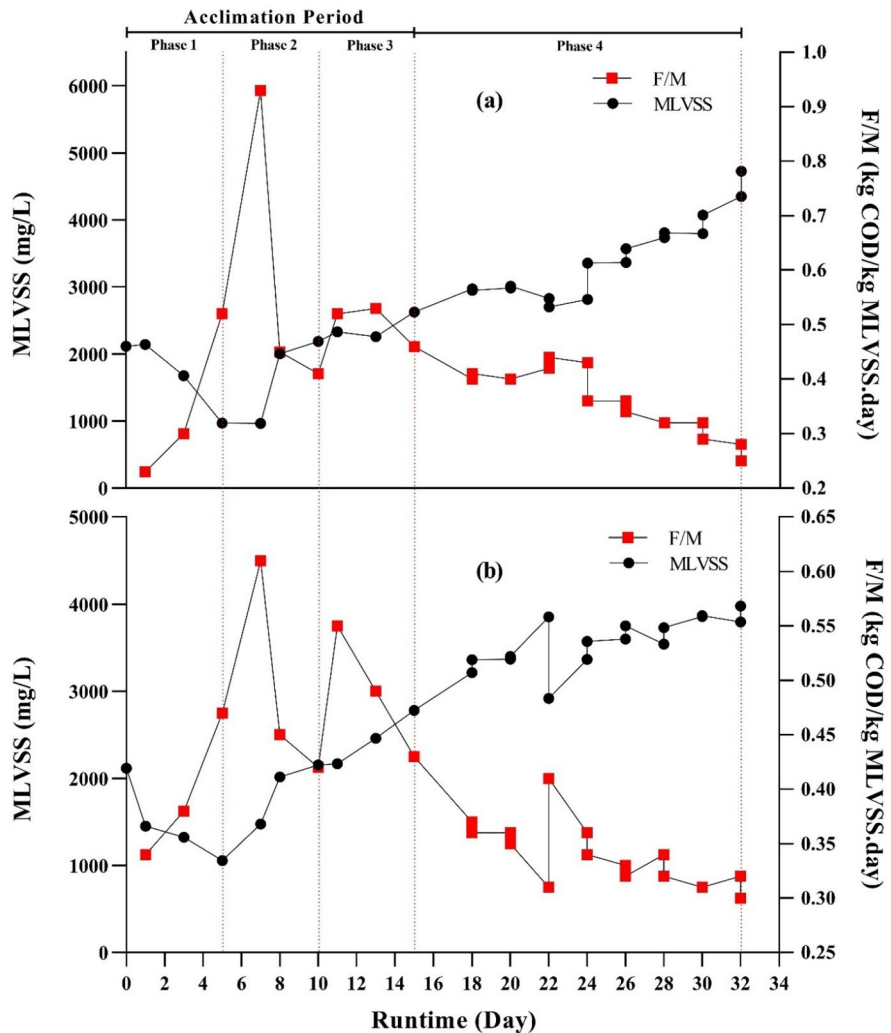
(P > 0.05) between the SBRs. The F/M ratio was calculated as 0.39 ± 0.0, 0.49 ± 0.1, 0.49 ± 0.0, and 0.33 ± 0.0 kg COD/kg MLVSS.day for each of the phases, respectively. Based on these results, it can be concluded that the gradual increase in the OLR did not cause any adverse effect in the stability of the sludge for both SBRs, and a sufficiently low F/M ratio allowed for the growth of stable sludge granules. The F/M values obtained for both reactors were in accordance with the F/M values of 0.33 kg COD/kg MLVSS.day obtained by Tay et al. (2004).

COD removal performance of the SBRs

COD removal efficiency in the SBRs and the mean value of the removal efficiency for each phase are

presented in Fig. 6 and Table 3. The COD removal performance of both reactors was observed, as expected, to be higher than 90% in all phases (Table 3). Similar high COD removal performances of aerobic SBRs were also reported by Zhou et al. (2013) and Hajiabadi et al. (2009). The result of the *T* test showed a significant difference between the SBRs during phase 2 (P = 0.005), phase 3 (P = 0.0211), and phase 4 (P < 0.0001). Oğuz (2005) observed that the examined nitrification inhibitors, ATU and azide, both inhibited the CO_2 fixation, and the inhibition during NO_2^- oxidation resulted in low CO_2 fixation, which was likely due to the reduced energy available. The result of the study carried out by Almomani et al. (2019) showed a correlation between the rate of CO_2 fixatiton and COD removal, and higher COD removal

Fig. 6 COD removal efficiency of the SBRs
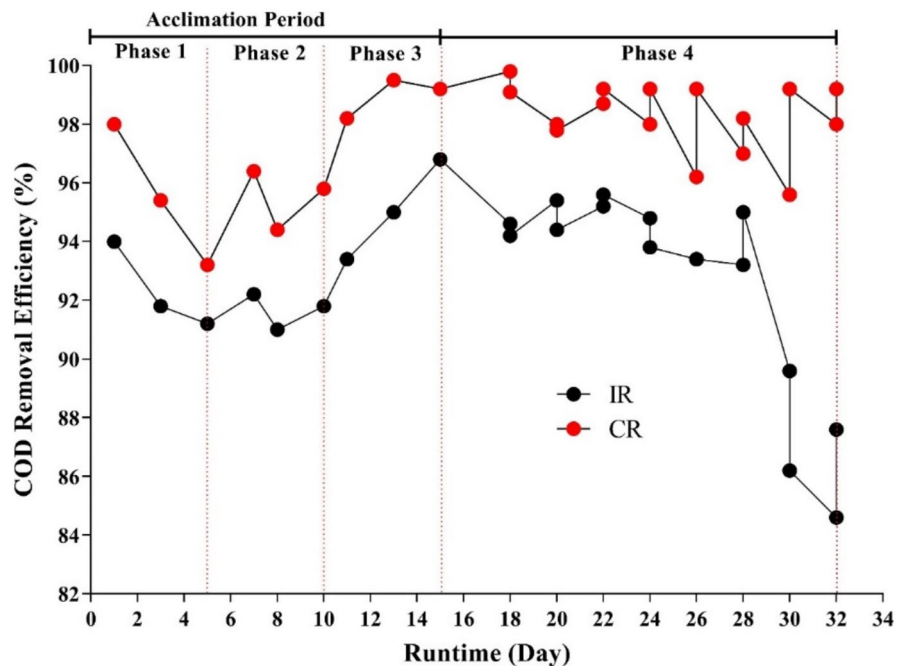
was obtained when the rate of CO_2 fixation was higher. In the current study, the reason for the higher COD removal in the CR compared to the IR may be the result of a higher rate of CO_2 fixation due to the higher availability of energy for the nitrifying bacteria existing under un-inhibited conditions in the CR. The results of the correlation analysis showed that the effluent COD concentration in the IR and CR was significantly correlated with the MLSS and MLVSS parameters (Table 4). The change of the sign of r values (from negative to positive) between the MLSS and effluent COD concentration for both reactors implied that the removal of COD in phase 4 affected positively by the gradually increasing MLSS concentration. A similar finding was also reported by Ren et al. (2005).

The differences between the performance of the SBRs in each phase were summarized based on the mean value and significance of difference (P value) in Table 3. It should be noted that the P values > 0.05 reveal that the model is non-significantly different, whereas values < 0.05 indicate that the model is highly significantly different, and there is a significant difference between variables based on a confidence level of 95% (Ahmadzadeh et al., 2017; Dolatabadi & Ahmadzadeh, 2019; Dolatabadi et al., 2021a, b; Yoosefian et al., 2017). According to the T test results, it was evidenced that there was no difference between

the SBRs in terms of the sludge settling performance, implying that the inhibition of nitrification did not result in any adverse effect on the sludge settling performance during the entire operational period (Fig. 7a). However, there was a slight difference between the SBRs in terms of the effluent TSS concentration in phase 4, resulting from the lower TSS concentrations in the cr compared to the ır (Fig. 7b). However, although there was a lower difference in terms of COD removal in phases 2 and 3, a significant difference was observed during phase 4, implying that the inhibition of nitrification remarkably affected the COD removal performance of the IR, possibly due to its effect on the reduced energy utilization rate of the nitrifying microorganisms.

Beun et al. (1999) examined the effect of different operational conditions on the aerobic granule formation, and carried out a series of trials in SBRs where the HRT value was set as 8 and 6.75 h. According to the results, it was reported that the reactor operation under the 8-h HRT condition was insufficient (in terms of time) to ensure the granulation of the biomass. During this operation, the sludge with poor settling performance was washed-out from the SBR. However, reducing the HRT to 6.75 h allowed to increase the stability of granular biomasses. With the reduction of the HRT value to 6.75 h, a new granulation phase started, and stable (without biomass

Fig. 7 Photo of (**a**) the sludge settling phase, and (**b**) the comparison of effluent of the SBRs with tap water
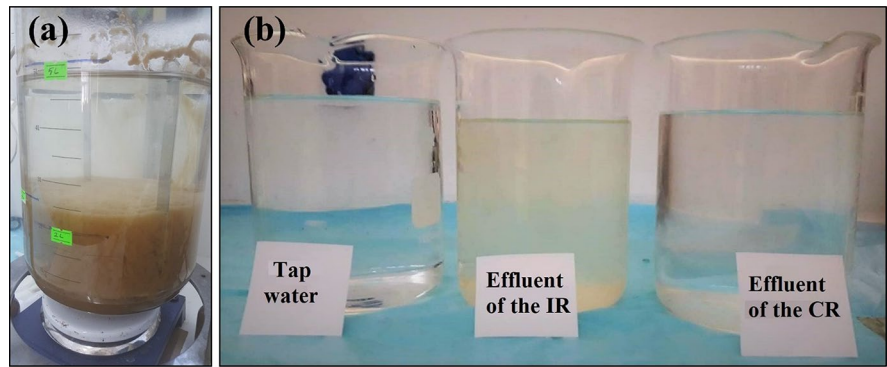
washing due to high sludge settling performance) filament-free granules started to form during the following operational stage. Similar findings were also obtained by Wang et al. (2005), investigating the effect of the HRT value on aerobic granular sludge formation in two identical reactors with 3 and 12 h of HRT values. It was determined that the SVI value decreased from 103 to 45 mL/g in the reactor, which was operated with 8 cycles per day under a 3-h HRT condition, at the end of the 16-cycle operating period. Contrary to this result, in the reactor operated under the 12-h HRT condition, it was determined that the sludge settling performance deteriorated at the end of the 16-cycle operation, the MLSS concentration decreased as a result of the biomass wash-out occurring during the following operation period, and the sludge settling performance was only improved after 22 days with the formation of small granules.

In the study conducted by Bindhu and Madhu (2013), the effect of OLR on the aerobic granular sludge formation was examined. According to the results obtained from the experiments carried out in three identical reactors with an OLR value of 3, 6, and 9 kg COD/m^3.day (the initial COD concentrations of 1000, 2000, and 3000 mg/L, respectively), it was found out that the granular properties of the sludge, and, thus, the reactor performances were improved until a certain level of increase in the OLR. In this study, the best reactor performance was achieved when the OLR was 6 kg COD/m^3.day, while increasing the load higher than that value caused fluctuations in COD removal and a decrease in MLSS concentration.

Gao et al. (2011) studied the effect of different operational conditions on the aerobic granular sludge formation in four separate SBRs. In each reactor, each

of the four experimental conditions (OLR, sludge settling time, aerobic cycle time, and air flow) was tested as a variable factor. According to the results, the aerobic granular sludge with superior settling performance was developed in the reactor having the initial sludge settling time of 15 min, which was gradually decreased to 1 min during 45 days of reactor operation. As a result of the study, it was reported that the most important factor affecting granular sludge formation among the examined variable factors was the settling time.

The operational strategies used during the acclimation period of the current study, which was determined by analyzing the findings explained in the abovementioned literature studies, allowed for accomplishing both of the reactor operations with a short sludge settling time (10 min), although the MLSS concentration increased gradually, and a high COD removal efficiency was observed during the whole experimental phase. In both reactors, an improvement in the sludge settling performance occurred during the entire operational period due to the dual-effect of reducing the HRT and increasing the OLR value by increasing the volume of the influent during the acclimation period. The daily shortening of the settling time by 1 min during the acclimation period also contributed to the increase of the reactor performances.

Evaluation of the effluent quality

Experimental values for the variations in the effluent PO_4^{3-}, NH_4^+, and NO_3^- concentrations in the SBRs, as well as the mean values and statistical analyses of data, are presented in Fig. 8 and Table 5, respectively. To better evaluate the effect of the inhibition of nitrification on the effluent quality, the removed NH_4^+

concentrations ($NH_4^+{}_{rem.}$) in the SBRs were used to estimate the rate of nitrification, which was calculated based on the ratio of the effluent NO_3^- concentration to the $NH_4^+{}_{rem.}$ (Fig. 9 and Table 6).

Effluent PO_4^{3-} concentration profile of the SBRs

Since EBPR requires alternating anaerobic and aerobic conditions (Wu et al., 2009), partial P removal might occur up to a certain level in an aerobic SBR system, which supports the observed results for both reactors. In the current study, the effluent PO_4^{3-} concentrations ranged between 4.6 and 19.6 mg/L for the IR, and 10.1–21.7 mg/L for the CR during phase 4 (Fig. 8a and Table 5). According to the mean effluent PO_4^{3-} concentrations (10.0 ± 4.4 mg/L for the IR and 17.0 ± 4.0 mg/L for the CR, Table 5), the mean removal efficiency was calculated as 64.9 and 40.4% for the IR and CR, respectively, based on the influent PO_4^{3-} concentration of 28.6 mg/L. Similarly, relatively low PO_4 removal performances ranging between 39 and 54% were also obtained in the aerobic SBR systems (Costa et al., 2013, 2019). The results of the current study showed that the mean values in the IR were lower than that of the CR in each operation phase due to the relatively higher P uptake by the microbial population (Table 5). The T test results revealed that there was no significant difference between the SBRs during the acclimation period ($P > 0.05$), while a significant difference was observed in phase 4 ($P < 0.0001$), implying that the inhibition of nitrification caused higher P uptake in phase 4 compared to the CR. This result was also supported by the study of Vogel et al. (2020) who showed that P uptake slightly increased in all three P fertilizer treatments with the nitrification inhibitors, which were added to enhance P plant availability in soils. The correlation analysis showed that there was no significant correlation between effluent PO_4^{3-} and the other considered parameters (Table 4).

Effluent NH_4^+ concentration profile of the SBRs

The variations in the effluent NH_4^+ concentration in the IR and CR are presented in Fig. 8b. In the IR, relatively higher effluent NH_4^+ concentrations were achieved during phase 4 compared to the CR. In this phase, the mean effluent NH_4^+ concentrations were

found to be 3.9 ± 2.4 and 1.26 ± 0.84 mg/L for the IR and CR, indicating that over threefold NH_4^+ ions remained in the effluent stream of the IR compared to that of the CR as a result of the inhibition treatment. The T test results showed that there was no significant difference between the SBRs during the acclimation period ($P > 0.05$), while a significant difference was obtained in phase 4 ($P = 0.0011$) (Table 5). These results clearly depict a difference between the SBRs. According to the results, it can be inferred that NH_4^+ ions were removed to form NO_2^- or NO_3^- ions due to the lack of inhibitory conditions in the CR together with its utilization as main N source by microbial population. However, the magnitude of the reduction in NH_4^+ concentration in the IR was lower in phase 4 due to the inhibitory conditions. Guo et al. (2021) and Liu et al. (2015) found that the nitrification inhibitors slowed down the nitrification process during incubation of the examined soil, which resulted in observing higher NH_4^+ concentrations as compared to the control samples. In the IR, the reason for the increase in the effluent NH_4^+ concentration after the OLR increase was the inhibition of nitrification, implying that the inhibition of nitrification caused higher P uptake in phase 4 compared to the CR. ATU has been reported to selectively inhibit ammonia oxidation at concentrations between 8 and 80 μM (Oğuz, 2005), and it has a mechanism which targets the ammonia monooxygenase action that chelates the copper in the active site, ultimately hindering its function (Tatari et al., 2017).

Effluent NO_2^- and NO_3^- concentration profile of the SBRs

The result of the effluent NO_2^- measurements showed that the mean concentration values for the IR and CR were 0.12 ± 0.0 and 0.11 ± 0.0 mg/L in phase 1, 0.10 ± 0.0 and 0.11 ± 0.0 mg/L in phase 2, 0.11 ± 0.0 and 0.13 ± 0.0 mg/L in phase 3, and 0.10 ± 0.0 and 0.23 ± 0.2 mg/L in phase 4 (data not shown). The result of the T test revealed that there was no significant difference between the SBRs in terms of the effluent NO_2^- concentration in each phase ($P > 0.05$), indicating that the inhibitor did not cause any significant inhibition on the activity of the NO_2^- oxidizing bacteria during the entire reactor operation. Oğuz (2005) studied the effect of the inhibition of ATU on NO_2^- removal and NO_3^- formation and found that,

Fig. 8 Variation of effluent PO_4^{3-} (**a**), NH_4^+ (**b**), and NO_3^- (**c**) concentrations of the SBRs
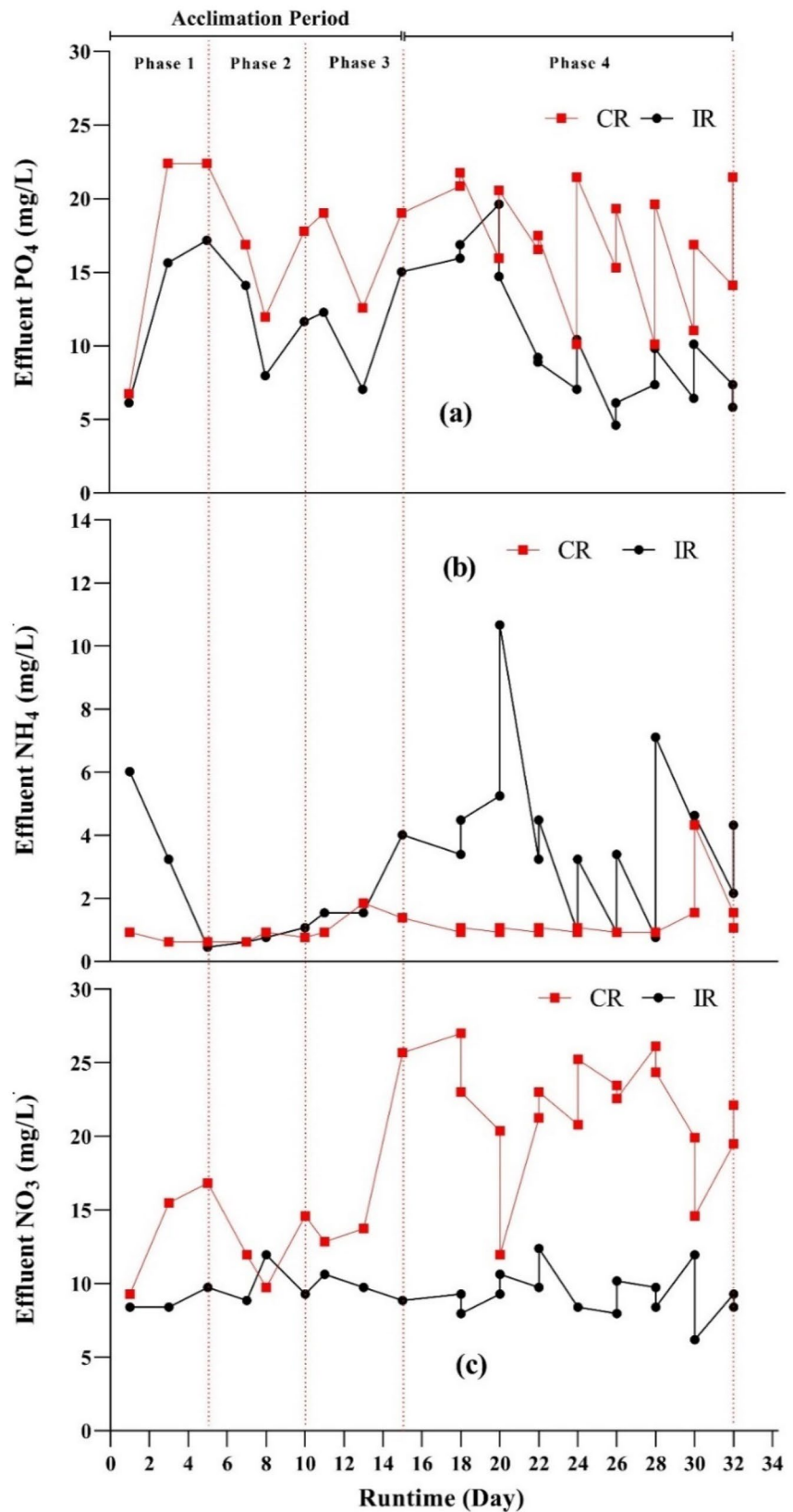
Table 5 Results of the T test analysis for the effluent quality parameters

Phase	Effluent quality parameters		Significance (P value)
	IR	CR	
	PO_4^{3-} (mg/L)		
1	12.9 ± 5.9	17.1 ± 9.0	Non-significant ($P > 0.05$)
2	11.2 ± 3.0	15.5 ± 3.1	Non-significant ($P > 0.05$)
3	11.4 ± 4.0	16.8 ± 3.7	Non-significant ($P > 0.05$)
4	10.0 ± 4.4	17.0 ± 4.0	Significant ($P < 0.0001$)
	NH_4^+ (mg/L)		
1	3.2 ± 2.7	0.72 ± 0.17	Non-significant ($P > 0.05$)
2	0.8 ± 0.2	0.77 ± 0.15	Non-significant ($P > 0.05$)
3	2.3 ± 1.4	1.39 ± 0.46	Non-significant ($P > 0.05$)
4	3.9 ± 2.4	1.26 ± 0.84	Significant ($P = 0.0011$)
	NO_3^- (mg/L)		
1	8.8 ± 0.7	13.8 ± 4.0	Non-significant ($P > 0.05$)
2	10.0 ± 1.6	12.1 ± 2.4	Non-significant ($P > 0.05$)
3	9.7 ± 0.8	17.4 ± 7.1	Non-significant ($P > 0.05$)
4	9.2 ± 1.5	21.5 ± 3.9	Significant ($P < 0.0001$)

depending on the experimental results, the inhibitor had no effect on the NO_2^- oxidation. In addition, statistical analysis also showed no significant difference in NO_2^- removal rates between the control and ATU tests. Based on the obtained results of the study, it was concluded that NO_2^- was oxidized to NO_3^- in both control and ATU samples, and that NO_2^- oxidizing bacteria in the ATU samples behaved similarly to NO_2^- oxidizing bacteria in the control samples, since ATU did not affect NO_2^- oxidation (i.e., NO_2^- removal $= NO_3^-$ formation).

The effect of the inhibition of nitrification was also investigated by considering the effluent NO_3^- concentrations (Fig. 8c and Table 5) and the other related control parameter, the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ (Fig. 9 and Table 6). As can be observed in Fig. 8c, the trend for effluent NO_3^- concentration in the SBRs was quite different. The results clearly showed that the effluent NO_3^- concentrations in the CR were generally higher than that of the IR during the complete operational period due to the lack of inhibition conditions. The mean concentrations for the IR and CR were found to be 8.8 ± 0.7 and 13.8 ± 4.0 mg/L in phase 1, 10.0 ± 1.6 and 12.1 ± 2.4 mg/L in phase 2, 9.7 ± 0.8 and 17.4 ± 7.1 mg/L in phase 3, and 9.2 ± 1.5 and 21.5 ± 3.9 mg/L in phase 4, respectively (Table 5).

According to the results of Pearson correlation analysis, no significant correlation between the effluent NO_3^- and NH_4^+ concentration was observed for both of the SBRs during the acclimation period (Table 4). However, a moderate significant negative correlation ($r = 0.53$, $P < 0.05$) was observed for the CR in phase 4. Correlatively, the T test results for the effluent NO_3^- concentration showed that there was no significant difference between the SBRs during the acclimation period ($P > 0.05$), while a significant difference was observed in phase 4 ($P < 0.0001$) (Table 5). A significant difference was also observed for the $NH_4^+{}_{rem.}$ ($P = 0.0011$) and the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ ($P < 0.0001$) only in phase 4 (Table 6). According to the experimental results, higher and almost a constant NH_4^+ removal took place in the CR as compared to the IR (Fig. 9a). However, the trend for the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ was quite different between the SBRs (Fig. 9b) and was similar to the trend obtained for the effluent NO_3^- concentration (Fig. 8c). The mean values for the $NH_4^+{}_{rem.}$ and the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ revealed that although the $NH_4^+{}_{rem.}$ values for both SBRs were similar in each phase, the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ increased gradually and ranged between 0.59 ± 0.1 and 0.94 ± 0.1 in the CR, while it ranged between 0.42 ± 0.0 and 0.47 ± 0.1 in the IR (Table 6). These results show that the rate of nitrification was two-fold higher in the CR during phase 4 as compared to the IR. In this phase, 94% of the $NH_4^+{}_{rem.}$ was converted to NO_3^- in the CR indicating an almost complete nitrification. On the other hand, only 47% of the $NH_4^+{}_{rem.}$ was converted to NO_3^- in the IR due to the inhibition of nitrification. Based on this result, it can be noted that the inhibition of nitrification took place at a rate of 53% in the IR, meaning a partial inhibition was obtained with the ATU treatment.

Lehtovirta-Morley et al. (2013) observed that ATU led to partial inhibition in liquid culture samples based on the final NO_2^- yield, giving significant inhibition of 85% when compared to control cultures in the presence of 100 μm ATU. Other examined inhibitors, DCD and nitrapyrin, resulted in complete inhibition of ammonia oxidation at the concentrations of 5 mM and 50 μm. Liu et al. (2015) studied the effect of two nitrification inhibitors, acetylene (C_2H_2) and 3,4-dimethylpyrazole phosphate (DMPP), on the nitrification and the ammonia oxidizer population in

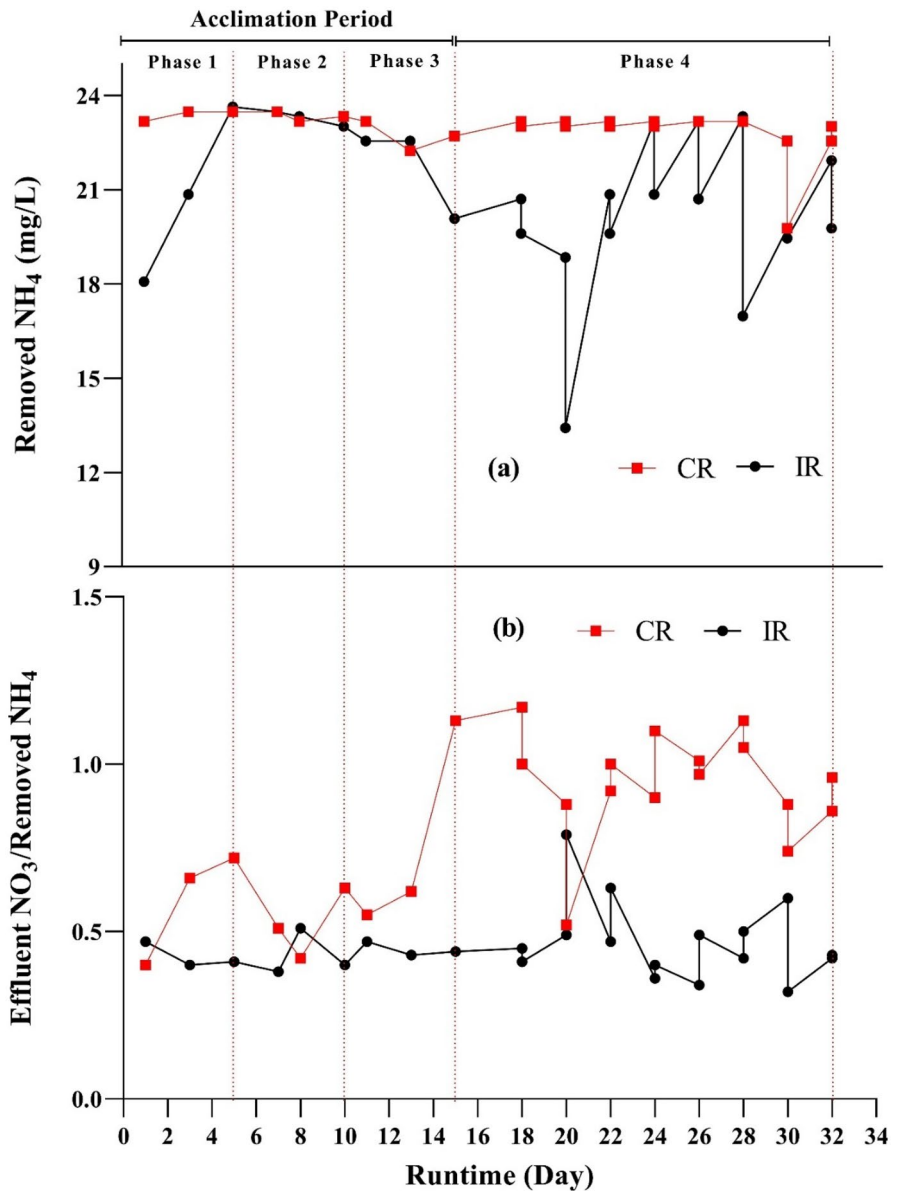**Fig. 9** Variations of the $NH_4^+{}_{rem.}$ (**a**) and the ratio of the effluent NO_3^- to $NH_4^+{}_{rem.}$ (**b**) of the SBRs

Table 6 Results of the *T* test analysis for the $NH_4^+{}_{rem}$ and the ratio of the effluent NO_3^- to $NH_4^+{}_{rem}$

	$NH_4^+{}_{rem.}$ (mg/L)			Effluent $NO_3^-/NH_4^+{}_{rem}$		
Phase	IR	CR	Significance (*P* value)	IR	CR	Significance (*P* value)
1	20.8 ± 2.7	23.3 ± 0.1	Non-significant ($P > 0.05$)	0.42 ± 0.0	0.59 ± 0.1	Non-significant ($P > 0.05$)
2	23.2 ± 0.2	23.3 ± 0.1	Non-significant ($P > 0.05$)	0.43 ± 0.0	0.52 ± 0.1	Non-significant ($P > 0.05$)
3	21.7 ± 1.4	22.7 ± 0.4	Non-significant ($P > 0.05$)	0.44 ± 0.0	0.76 ± 0.3	Non-significant ($P > 0.05$)
4	20.1 ± 2.4	22.3 ± 0.8	Significant ($P = 0.0011$)	0.47 ± 0.1	0.94 ± 0.1	Significant ($P < 0.0001$)

alkaline, neutral, and acidic soils. The result of the study showed that C_2H_2 completely inhibited the production of NO_3^- in all soils compared to control samples, while DMPP completely inhibited the production of NO_3^- in neutral soils and ultimately slowed the NO_3^- formation in the other soils. Additionally, the NH_4^+ concentrations in the treated soils were found to be higher than that of the control samples. According to the overall results, it was reported that the two inhibitors significantly suppressed the activity of the ammonia-oxidizing microorganisms and decreased NO_3^- content by 57–85% in the neutral

soil. Grant et al. (2020) and Guo et al. (2021) reported similar results, indicating that the examined nitrification inhibitors resulted in slowing the nitrifier growth and caused an increase in soil NH_4^+ concentrations and a decrease in soil NO_3^- concentrations. The effect of the inhibition treatment using ATU in this study has been compared with that of various nitrification inhibitors in Table 7. It can be understood from the given literature examples that the effectiveness of a nitrification inhibitor is significantly affected by the type and concentration of inhibitor as well as the type of the examined sample.

Table 7 Comparison of the effect of the inhibition treatment of this study with other inhibitors from the literature at the corresponding conditions of inhibitor concentration

Inhibitor	Concentration	Sample	Effect of the inhibition treatment	Reference
Acetylene (C_2H_2)	1% of the headspace of 500-mL vial	Soil (including NH_4Cl of 100 μg N/g soil)	97.1 to 100% inhibition based on the type of soil sample	Liu et al. (2015)
ATU	100/500/1000 nmol per gram soil	Soil	No detectable inhibition for 100 nmol ATU 24%/26% inhibition for 500/1000 nmol ATU	Lehtovirta-Morley et al. (2013)
	10/50/100 μM	Liquid culture	29%/63%/85% inhibition for 10/50/100 μM ATU	
	86 μM	Primary clarifier effluent	No significant inhibition	Oğuz (2005)
	70 μm per Liter feeding solution	Synthetic wastewater	53% inhibition	This study
Dicyandiamide (DCD)	1/5 μmol per gram soil	Soil	25%/100% inhibition	Lehtovirta-Morley et al. (2013)
	1/5/10 mM	Liquid culture	33% inhibition for 1 mM DCD Complete inhibition for 5/10 mM DCD	
	5% of the applied NH_4-N content of 25 mg/kg soil	Soil	33 to 86% decrease in the total N_2O-N emission depending on the type of soil sample	Guo et al. (2021)
3,4-Dimethylpyrazole phosphate (DMPP)	29% per gram NH_4Cl solution	Soil (including NH_4Cl of 100 μg N/g soil)	70.5 to 93.5% inhibition depending on the type of soil sample	Liu et al. (2015)
	0.6% (w/w)	Horticultural soil	> 50% reduction in N_2O emission	Friedl et al. (2020)
	5% of the applied NH_4-N content of 25 mg/kg soil	Soil	30 to 88% decrease in the total N_2O-N emission depending on the type of soil sample	Guo et al. (2021)
Nitrapyrin	10/50 μM	Liquid culture	92%/100% inhibition	Lehtovirta-Morley et al. (2013)

Conclusions

To ensure the sustainable use of resources such as P and N, which are utilized during the production of fertilizers and, thus, consumed in high amounts in agricultural applications all around the world, the topic on the nutrient recovery in WWTPs has attracted great attention in recent years. In this study, the effect of the inhibition of nitrification on the reactor performance and effluent quality was investigated in aerobic SBRs, with the aim of suppressing the formation NO_x and, consequently, increasing the NH_4^+ concentration in the effluent streams to create an increased potential for nutrient recovery from the effluent streams of aerobic SBRs. According to the results presented, the operational strategies applied during a short-term acclimation period allowed to operate the SBRs with the high treatment performance in terms of removal of COD (over 92% of removal efficiency) and TSS (< 50 mg/L) with the help of fast settling AS (SVI < 60 mL/g) although gradually increasing MLSS concentration. Furthermore, the inhibition treatment did not affect the main physicochemical properties of the AS such as growth of microbial population (MLSS), SRT, and F/M adversely.

The results obtained from the measurements for the effluent quality showed that the inhibition of nitrification resulted in achieving lower PO_4^{3-} (10.0 ± 4.4 mg/L), over threefold higher NH_4^+ (3.9 ± 2.4 mg/L), and over twofold lower NO_3^- (9.2 ± 1.5 mg/L) concentrations in the effluent compared to the un-inhibited conditions. While the rate of nitrification during un-inhibited conditions was found to be 0.94 ± 0.1, representing almost a complete nitrification, it was found to be 0.47 ± 0.1 under inhibitory conditions, implying a partial inhibition. The results clearly demonstrated that the inhibition of nitrification increased the effluent NH_4^+ concentrations by suppressing the formation of NO_3^- ions. However, it should be noted that the obtained results for the effluent NH_4^+ concentrations were not very satisfactory, decreasing the potential for recovery of nutrients depending on the examined conditions. In future studies, to further increase the potential, it is highly recommended to (1) operate reactors for a longer period of time under controlled growth of the microbial population (e.g., lower MLSS concentration), (2) increase the efficiency of inhibition of nitrification by testing different inhibitors, and (3) investigate the effect of different substrate sources and operating parameters on the effluent quality.

The present study provides new insights into the requirement of inhibiting the nitrification in an aerobic SBR system to increase the potential for the recovery of NH_4^+ together with PO_4^{3-} from effluent discharges of WWTPs through a suitable recovery process such as struvite precipitation from the effluent discharges. The idea of inhibiting the nitrification step may inspire new research studies in this topic. The ability to recover and reuse nutrients from waste streams could reduce dependence on other more energy-intensive processes that are currently being used to synthesize fertilizer products, and instead reduce the dependence on mined, finite mineral resources. It would also provide municipalities the opportunity to create a revenue source to replace costly nutrient removal endeavors. In conclusion, mitigating the negative impacts that might occur in social, environmental, and economic areas can allow local governments to move towards a sustainability approach with wastewater treatment.

Declarations

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