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NITROGEN REMOVAL FROM AMMONIUM-RICH PHARMACEUTICAL WASTEWATER. A COMPARISON BETWEEN SEQUENCING BATCH REACTOR (SBR) AND SEQUENCING BATCH BIOFILM REACTOR (SBBR)

Biological nitrogen removal from industrial wastewater (pharmaceutical synthetic wastewater) was studied. Sequencing batch reactor (SBR) and sequencing batch biofilm reactor (SBBR) were utilized to treat ammonium-rich wastewater. No external carbon source was added to the reactors. At nitrogen loading rate (NLR) of 2.5 kg N/(m³·day), the efficiency of ammonium removal from synthetic wastewater in SBR was 86.9%. SBBR ammonium removal efficiency under fixed dissolved oxygen (DO) concentration of 0.3 mg O₂/dm³ was 98.4%. Specific nitrification rate (SNR) and specific denitrification rate (SDNR) in SBR were 9.24 mg NH₄⁺-N/(g MLVSS·h) and 18.56 mg NO₃⁻-N/(g MLVSS·h), respectively. Nitrite accumulation rate (NAR) showed high correlation with DO decrement ($R^2 = 0.983$), NAR and simultaneous nitrification and denitrification (SND) had high correlation, too ($R^2 = 0.976$). SND efficiency in SBBR reached 94.1% while ammonium oxidizing bacteria (AOB) were dominant and NAR in SBBR process at the DO level of 0.3 mg O₂/dm³ was 90.3%.

ABBREVIATIONS USED IN THE TEXT

AOB	– ammonium oxidizing bacteria
BOD	– biological oxygen demand
COD	– chemical oxygen demand
DO	– dissolved oxygen
DR	– denitrification ratio
HRT	– hydraulic retention time

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MLSS	– mixed liquor suspended solids
MLVSS	– mixed liquor volatile suspended solids
NAR	– nitrite accumulation rate
NLR	– nitrogen loading rate
NOB	– nitrite oxidizing bacteria
NR	– nitrification ratio
OLR	– organics' loading rate
ORP	– oxidation-reduction potential
SBBR	– sequencing batch biofilm reactor
SBNR	– shortcut biological nitrogen
SBR	– sequencing batch reactor
SDNR	– specific denitrification rate
SND	– simultaneous nitrification and denitrification
SNR	– specific nitrification rate
SRT	– solids' retention time
SS	– suspended solids
TKN	– Kjeldahl nitrogen
TN	– total nitrogen
VSS	– volatile suspended solids

1. INTRODUCTION

Ammonium ($\text{NH}_4^+\text{-N}$) is main nitrogen compound in the most types of municipal or industrial wastewater. Preliminary treatment of wastewater by using screens, removes large pieces of trash. Primary treatment of wastewater removes suspended solids in settling tanks, while secondary treatment (biological treatment) of wastewater removes organic materials. Tertiary or advanced wastewater treatment improves the effluent quality to meet the standard of discharge concentration. Nutrients removal occurs during secondary treatment. Therefore, ammonium removal systems can be advanced or conventional. At $\text{pH} > 8.5$, ammonia ($\text{NH}_3\text{-N}$) is formed but ammonium is the main nitrogen compound of wastewater at normal pH ranges [1]. Other wastewater nitrogen compounds such as nitrate ($\text{NO}_3^-\text{-N}$) and nitrite ($\text{NO}_2^-\text{-N}$) are known as water bodies contaminants but the concentration of these nutrients is low in raw wastewater. Another wastewater nitrogen compound is organic nitrogen (Org-N). The sum of Org-N and ammonium is known as total Kjeldahl nitrogen (TKN). Total nitrogen (TN) is the sum of TKN and nitrogen oxides (nitrate and nitrite). Therefore, the concentration of TN in wastewater is close to TKN. Entering effluent nitrogen compounds in lakes may cause problems like eutrophication and dissolved oxygen (DO) depletion [2].

Some types of industrial wastewater, for example, pharmaceutical, petrochemical or manure production wastewater has high TN. Ammonium level could be high in such a wastewater called ammonium-rich wastewater or high-strength ammonium wastewater [3]. Physical, chemical or biological methods would be utilized to treat ammonium-rich wastewater. Physical or chemical methods have high removal efficiency but they

are expensive processes. Biological methods have high nitrogen removal efficiency [4]. Biological processes are cost-beneficial and easy to operate but the feasibility of using these treatment methods should be checked for pharmaceutical or petrochemical wastewater treatment [5]. A number of modified approaches to biological nitrogen removal includes completely autotrophic nitrogen removal over nitrite (CANON), anaerobic ammonium oxidation (ANAMMOX), oxygen-limited autotrophic nitrification-denitrification (OLAND), simultaneous nitrification and denitrification (SND) via nitrite, simultaneous nitrification-anammox-denitrification (SNAD), single reactor system for high activity ammonium removal over nitrite (SHARON) and deammonification (DEMON) [2, 6].

The sources of pharmaceutical wastewater include: synthesized organic materials industries (1), fermentation industries (2), biological production industries such as serums production (3), industries of mixing and formulation of drugs (4). Biological methods are not proper to treat the wastewater of synthesized organic materials industries. Chemical oxygen demand (COD) is high in such a wastewater but when the ratio of biochemical oxygen demand (BOD) to COD is not low, biological methods could be utilized. BOD in the wastewater of fermentation industries is high and biological methods could be used. The wastewater of biological production industries such as serums production contains high level of nitrogen and, furthermore, its BOD and COD are high. The wastewater of industries of mixing and formulation of drugs is high in BOD, low in suspended solids (SS) and may be toxic [7].

Sequencing batch reactor (SBR) and sequencing batch biofilm reactor (SBBR) are two methods for nitrogen removal with high efficiency. SBBR could be made by adding fixed biofilm carriers to SBR. Upgrading SBR to SBBR would be an interesting method to increase nutrient removal efficiency in wastewater treatment plants (WWTP). SBBR provides higher nitrogen removal efficiency through shortcut biological nitrogen removal (SBNR). Many researchers studied nitrogen removal by SBR and SBBR [8, 9] but a few of them surveyed the comparison between nitrogen removal from ammonium-rich wastewaters by SBR and SBBR [10]. Some of researchers studied nitrogen removal from wastewater with low COD or low carbon to nitrogen (C/N) ratio [3, 11].

A number of authors focused on two-sludge systems for ammonium-rich wastewater treatment. In such systems, nitrifiers and denitrifiers are separated completely in two reactors [12] and a number of them combined two wastewaters with high nitrogen and high COD before ammonium removal from high-strength ammonium wastewater [13]. In this research a comparison between nitrogen removals from ammonium-rich wastewater with high COD (pharmaceutical wastewater of group 3) by SBR and SBBR has been done. Nitrogen removal requires sequent aerobic and anoxic phases to achieve nitrification and denitrification, respectively.

There are two methods for nitrogen removal by using SBR. In a single reactor subsequent aerobic and anoxic phases can be provided. Timing and adjusting the operational conditions are difficult in this method. Aerobic and anoxic conditions can be

achieved by using two subsequent tanks. The operation in this method is easier than the operation in a single reactor but it costs too much. SBBR process provides aerobic and anoxic conditions in a single reactor at the same time. In SBBR process anoxic and aerobic conditions are provided in the inner and outer layers of the biofilm, simultaneously. Further, the efficiency of nitrogen removal in SBBR is higher than SBR. In current study SBR operation was based on conventional nitrification (aerobic) and denitrification (anoxic) while SND via nitrite was provided in SBBR under aerobic condition. Upgrading SBR to SBBR would reduce operational costs of a wastewater treatment plant including energy, oxygen and external carbon source costs [14]. Further, as explained the number of required tanks in SBBR process is less than SBR because nitrogen compounds removal occurs, simultaneously.

2. MATERIALS AND METHODS

SBR and SBBR. Two 20 dm³ cylindrical plexiglas tanks were used as SBR and SBBR while 30% of the SBBR tank was filled with high density polyethylene (HDPE) biofilm carriers (Fig. 1).

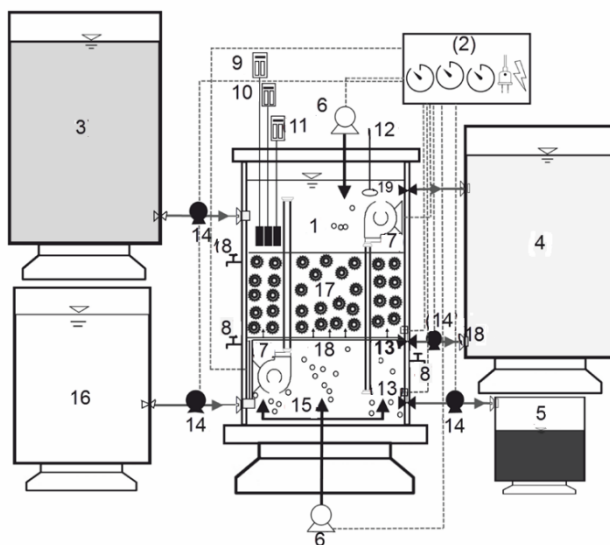


Fig. 1. Schematic diagram of SBBR:

- 1 – reactor, 2 – analog timer, 3 – influent tank, 4 – effluent tank, 5 – excess sludge tank, 6 – aerator,
- 7 – water pump, 8 – sampler, 9 – ORP meter, 10 – pH meter, 11 – DO meter, 12 – heater,
- 13 – solenoid valve, 14 – pump, 15 – air stone, 16 – backwash tank,
- 17 – fixed media, 18 – backwash tubes, 19 – backwash outlet valve

Packing media (Kaldnes K2, Noavaran Pakan Ghatreh Engineering Co., Iran), was fixed at the height of 20 cm from the bottom of SBBR. Table 1 shows the properties of the media. The bed was fixed between two plates which had several holes. Aeration of SBR and SBBR was provided simultaneously from top and bottom, and each reactor contained two water pumps for wastewater recirculation.

Table 1

Properties of Kaldnes K2

Property	Value
Specific weight, N/m ³	87±3
Dimensions (diameter×height), mm	19×14±2
Media working volume/media total volume, %	23
Material	HDPE
Effective surface per a media piece, cm ²	24.4±10
Nominal surface per a media piece, cm ²	30.06±10
Effective specific surface area, m ² /m ³	369.6
Nominal specific surface area, m ² /m ³	480±30

Synthetic wastewater. Synthetic wastewater was provided to simulate pharmaceutical wastewater with high ammonium content and COD but pharmaceutical compounds removal was not the subject of present study.

Table 2

Components of synthetic wastewater [mg/dm³]

Parameter	Concentration
BOD	6700–6800
COD	10000–10050
BOD/COD	0.670–0.676
NH ₄ ⁺ -N	1875–2000
NO ₃ ⁻ -N	75–83
NO ₂ ⁻ -N	30–33
Org-N	625–640
TKN	2630–2792
TP	20–23
Oil and grease	17–19
TOC	8135.3–8227

8 g of dry milk, 1.125 g of sodium acetate (C₂H₃NaO₂), 0.625 g of urea (CH₄N₂O), 2.1 g of ammonium nitrate (NH₄NO₃), 0.013 g of dipotassium hydrogen phosphate (K₂HPO₄), 0.01 g of potassium dihydrogen phosphate (KH₂PO₄), 0.8 g of sodium bicarbonate (NaHCO₃), 11 mg of magnesium sulfate heptahydrate (MgSO₄·7H₂O), 1 mg of

calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 1 mg of iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 0.084 g of chloramphenicol ($\text{C}_{11}\text{H}_{12}\text{O}_5\text{N}_2\text{Cl}_2$), 0.025 g of diclofenac ($\text{C}_{14}\text{H}_{10}\text{O}_2\text{NCl}_2\text{Na}$), 0.65 g of salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$), 0.14 g of paracetamol ($\text{C}_8\text{H}_9\text{O}_2\text{N}$), 0.07 g of nitrobenzene ($\text{C}_6\text{H}_5\text{NO}_2$) and 0.15 g of benzoic acid ($\text{C}_7\text{H}_6\text{O}_2$) were added to 1 dm³ of distilled water to make synthetic ammonium-rich wastewater. Physicochemical characteristics of synthetic pharmaceutical wastewater (Table 2) were based on past researches [15].

Reactor start-up and operation. The biomass (6 dm³ of seed) was delivered from a West Ahvaz wastewater treatment plant. At the end of the start-up, concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were 4113 and 3800 mg/dm³, respectively. Microbial adaptation with new environment in SBR and SBBR was achieved in 2 months and 4 months, respectively. When adequate biofilm was formed on the fixed media, treatment process in SBBR was started at pH of 7. Sodium bicarbonate was used to fix pH in the influent. Mixed culture of microorganisms was used so that ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) were present in the medium. Treatment process was started at low nitrogen loading rate (NLR) and low organic loading rate (OLR). Then, NLR and OLR were slightly increased.

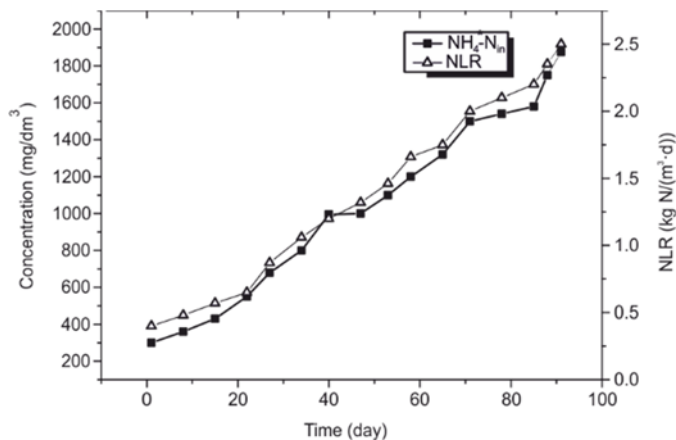


Fig. 2. Increasing influent $\text{NH}_4^+\text{-N}$ and NLR during start-up operation

Figure 2 shows increasing NLR until reaching required amount during start-up operation. NLR and OLR calculated according to the following equations was increased to reach 2.5 kg N/(m³·d), and 10 kg COD/(m³·d), respectively

$$\text{OLR} = \frac{Q_{\text{in}} S}{V} \quad (1)$$

$$\text{NLR} = \frac{Q_{\text{in}} N}{V} \quad (2)$$

where: OLR – organic loading rate, kg COD/(m³·d), NLR – nitrogen loading rate, kg N/(m³·d), Q_{in} – influent flow rate, m³/d, S – concentration of influent organic substrate, kg COD/m³, N – concentration of influent nitrogen, kg N/m³, V – reactor volume, m³.

The C/N ratio was 4, hydraulic retention time (HRT) 24 h, and temperature 20 °C. Solids' retention times (SRT) in SBR and SBBR were 25 days and 55 days. The operation of SBR and SBBR were carried out during two runs. SBR cycle and SBBR cycle were similar in run 1 including filling (5 min), aerobic phase (1360 min), settling (60 min) and drawing (15 min). A manual valve was used for filling the reactor from the influent synthetic wastewater reservoir. The filling event period was short to avoid continues mode in the sequencing batch reactor process. When the manual valve was open, a water pump was utilized for wastewater introduction into the test reactor. The number of filling events of SBR effects on nitrogen removal efficiency. It was reported that more filling events resulted in higher nitrogen removal efficiency [2] Therefore, one filling event in each cycle was considered to study other parameters that influence nitrogen removal efficiency, for example, DO concentration. SBBR was operated at fixed DO level of 1, 0.5 and 0.3 mg O₂/dm³ in run 1. An anoxic phase and an internal carbon source addition was considered during SBR cycle in run 2 to reduce effluent nitrate. Organic carbon materials as internal carbon source can be obtained from influent wastewater or endogenous carbon source (stored carbon materials within the cells). In the current study, 6 dm³ of influent synthetic wastewater was utilized as internal carbon source. Internal carbon source was introduced into SBR under anoxic condition at 10th hour of the cycle in run 2. Adding internal carbon source was similar to filling the reactor which described above. SBBR was operated at decreasing DO level from 1 to 0.5 mg O₂/dm³ and then from 0.5 to 0.3 mg O₂/dm³ in run 2. Regression analysis was conducted in each step of the study.

Analytical methods. BOD, COD, SS, VSS, NH₄⁺-N, NO₃⁻-N and NO₂⁻-N concentrations were analyzed according to standard methods for the examination of water and wastewater [16]. TKN was measured by the colorimetric method with preliminary distillation/digestion [17]. Org-N was determined from the difference between TKN and NH₄⁺-N. Each test was repeated 4 times and the average was reported as the result. Nitrogen compounds analysis was done after filtering the sample by a Whatman® membrane filter (pore size of 0.45 µm). Oxidation-reduction potential (ORP) and pH were measured using a portable multifunction device (HANNA instruments, Model, HI 991003, U.S.A). DO was determined using a portable device (WTW GmbH, Model, ProfiLineOxi 3210, Germany).

Nitrification and denitrification. Nitrification and denitrification in SBR could be studied using parameters such as nitrification ratio (NR), denitrification ratio (DR), specific nitrification rate (SNR) and specific denitrification rate (SDNR). NR, DR, SNR and SDNR could be determined from equations 3-6 but SNR and SDNR were analyzed in this study because these parameters are more complete

$$NR = \frac{NH_4^+ - N_{in} - NH_4^+ - N_{out}}{t} \quad (3)$$

$$SNR = \frac{NR}{MLVSS} \quad (4)$$

$$DR = \frac{NO_x^- - N_{in} - NO_x^- - N_{out}}{t} \quad (5)$$

$$SDNR = \frac{DR}{MLVSS} \quad (6)$$

where: NR – nitrification ratio, mg/(dm³·h), NH₄⁺-N_{in} – influent concentration of ammonium, mg/dm³, NH₄⁺-N_{out} – effluent concentration of ammonium, mg/dm³, *t* – time, h, SNR – specific nitrification rate, mg NH₄⁺-N/(g MLVSS·h), MLVSS – mixed liquor volatile suspended solids, mg/dm³, DR – denitrification ratio, mg/(dm³·h), NO_x⁻-N_{in} – influent concentration of nitrate plus nitrite (considering anoxic phase), mg/dm³, NO_x⁻-N_{out} – effluent concentration of nitrate plus nitrite (considering anoxic phase), mg/dm³, SDNR – specific denitrification rate, mg NO₃⁻-N/(g MLVSS·h).

SND via nitrite was studied in SBBR. The efficiency of SND was determined from the equation

$$SND \text{ efficiency} = \frac{NH_4^+ - N_{in} - NO_x^- - N_{out}}{NH_4^+ - N_{in}} \times 100\% \quad (7)$$

The nitrite accumulation rate (NAR) defined as follows was analyzed in SBBR

$$NAR = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N} \times 100 \quad (8)$$

where: NO₂⁻-N – effluent concentration of nitrite, mg/dm³, NO₃⁻-N – effluent concentration of nitrate, mg/dm³.

3. RESULTS AND DISCUSSION

3.1. EFFECT OF FIXED DO IN RUN 1

SBR was operated without anoxic phase (at the DO level of 1 mg O₂/dm³) and SBBR operated at fixed DO levels of 1, 0.5 and 0.3 mg O₂/dm³ in run 1. SBR and SBBR were operated at NLR of 2.5 kg N/(m³·d) and OLR of 10 kg COD/(m³·d). The results showed that the efficiency of SBR for TN removal was 71.5% in run 1. SBR removed 81% of pharmaceutical wastewater ammonium. Without anoxic phase in the cycle of the reactor or without adding fixed bed to SBR, ammonium was converted to nitrate but nitrate was not converted to nitrite. SNR was 8.73 mg NH₄⁺-N/(g MLVSS·h) in run 1 because no pre-anoxic or post-anoxic step was considered in the reactor cycle. SND in SBBR was achieved in run 1, successfully. SND efficiency at fixed DO concentrations of 1, 0.5 and 0.3 mg O₂/dm³ were 83.8, 90.9 and 94.1%, respectively. The SND efficiency was higher at lower DO concentrations. SND had correlation with the DO decrement ($Y = -0.068x + 6.725$, $R^2 = 0.999$) because the activity of NOB was limited while the activity of AOB was normal by DO adjustment. Maximum ammonium removal efficiency was 98.4% at fixed DO level of 0.3 mg O₂/dm³.

3.2. EFFLUENT NO_x⁻-N

The effluent nitrate concentration of SBR aerobic process was high during run 1. SBR effluent nitrate concentration of was 356.2 NO₃⁻-N/dm³ which did not meet the standards recommended by the Department of Environmental Protection Organization of Iran (DOE) [18]. Figure 3a shows the evolution of nitrogen compounds during a typical cycle of SBR in run 1. The nitrate concentration was increasing until the end of the cycle. The effluent nitrate concentration in SBBR in run 1 was lower than SBR because micro-environments were provided due to fixed biofilm carriers. Micro-environments provide anoxic zones along with aerobic zones for the activity of autotrophic and heterotrophic bacteria. Adequate depth of the biofilm provided both aerobic and anoxic zones to achieve SND via nitrite. Weekly backwash avoided adding sheared biofilm to MLSS and MLVSS. MLSS and MLVSS were kept in the range of 4050 to 6850 mg/dm³ and 3700 to 5100 mg/dm³, respectively. Fixing MLSS in a desired range was important for SRT evaluation. Further, maintaining MLVSS in a desired range was important to calculate SNR and SDNR.

Concentration of SBBR effluent nitrate at fixed DO level of 0.3 mg O₂/dm³ was 40 mg NO₃⁻-N/dm³ which met the standard of DOE. Figures 3b–d show the evolution of nitrogen compounds during a typical cycle of SBBR at various DO concentrations during run 1. Nitrite accumulation was higher at DO concentration of 0.3 mg O₂/dm³. Four enzymes are involved in denitrification. These are nitrate reductase (NAR and/or NAP), nitrite reductase (NirK or NirS), nitric oxide reductase (cNOR or qNOR) and

nitrous oxide reductase (N_2OR). Nitrite accumulation increment meant imbalanced enzymatic reactions at fixed DO level [19]. It was suggested that oxygen transfer limitation and long SRT were the main reasons of this phenomenon. Nitrite accumulation rate in SBBR process at the DO level of 1, 0.5 and 0.3 mg O_2/dm^3 were 70.2, 87 and 90.3%, respectively.

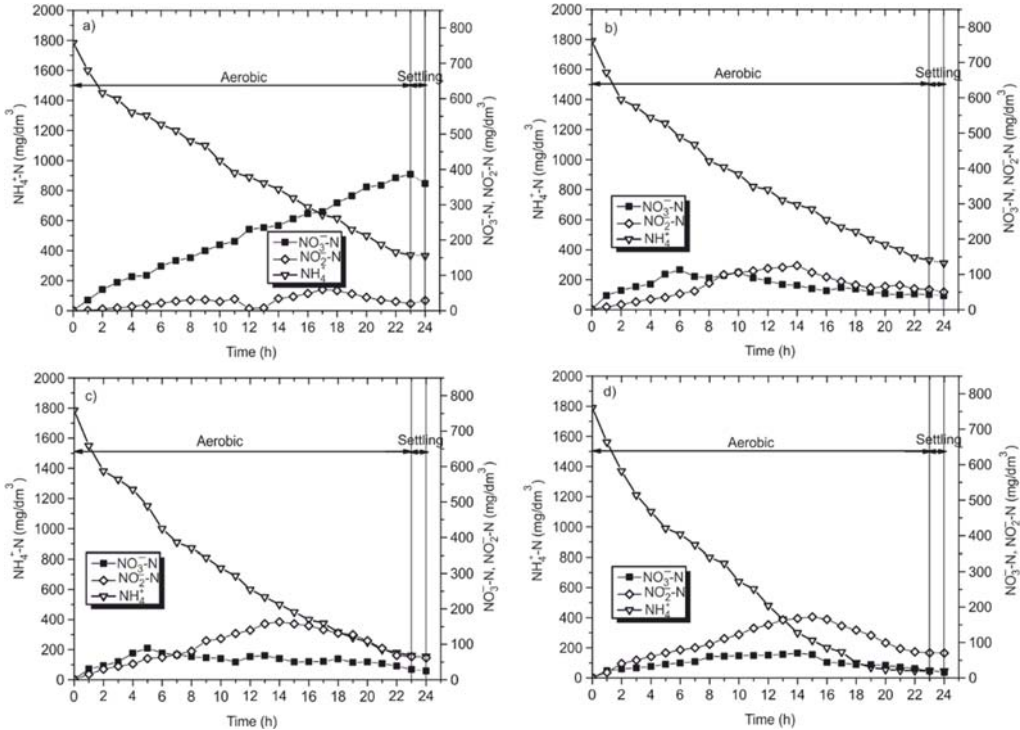


Fig. 3. Evolution of nitrogen compounds during a typical cycle of SBR and SBBR in run 1: a) SBR, b) SBBR at the DO level of 1 mg/dm³, c) SBBR at the DO level of 0.5 mg/dm³, d) SBBR at the DO level of 0.3 mg/dm³

During SBBR process in run 1, NAR was increased step-wise from the operation in which DO level was 1 mg O_2/dm^3 to the operation in which DO level was 0.3 mg O_2/dm^3 . This increment was happened because of high correlation of NAR with DO decrement ($y = -0.033x + 3.337$, $R^2 = 0.983$) which is shown in Fig. 4. Also, it is seen in Fig. 5 that when NAR displayed an increment, simultaneous removal of ammonium and nitrate was increased ($y = 2.020x - 98.52$, $R^2 = 0.976$) during run 1. Figure 6 describes differences between TN removal efficiency and SND efficiency. In Figure 6, sections a, b and c indicate the operation of SBBR during run 1 at the DO level of 1, 0.5 and 0.3 mg O_2/dm^3 , respectively. As demonstrated in Fig. 6, there was a difference of more than 10% between TN removal and SND efficiency in run 1 at the DO level of 1 mg O_2/dm^3

(section a) but decreasing DO led to close efficiencies of TN removal and SND in SBBR process at DO concentration of $0.3 \text{ mg O}_2/\text{dm}^3$ (section c). Figure 7 shows $\text{NO}_x\text{-N}$ concentrations in the effluent of SBR and SBBR in run 1 and run 2 during 30 days of the effluent parameters analysis. Generally, the effluent $\text{NO}_x\text{-N}$ concentration in SBBR was lower than that in SBR because the biofilm depth acted as an anoxic zone to remove $\text{NO}_x\text{-N}$. Minimum effluent $\text{NO}_x\text{-N}$ concentration of SBBR was observed at a fixed DO concentration of $0.3 \text{ mg O}_2/\text{dm}^3$.

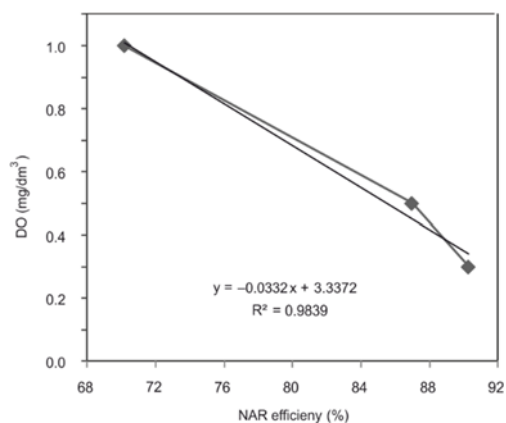


Fig. 4. The correlation between NAR and DO decrement during SBBR process in run 1

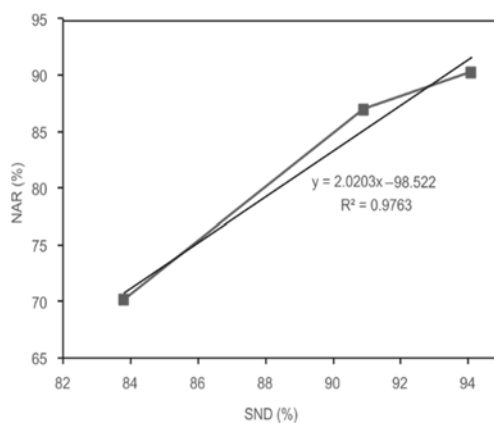
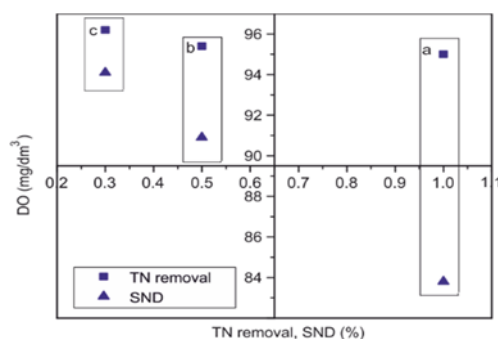


Fig. 5. A correlation between NAR and SND during SBBR process in run 1

Fig. 6. TN removal efficiency and SND efficiency in SBBR during run 1:
section a – SBBR at the DO level of $1 \text{ mg O}_2/\text{dm}^3$,
section b – SBBR at the DO level of $0.5 \text{ mg O}_2/\text{dm}^3$, section c: SBBR at the DO level of $0.3 \text{ mg O}_2/\text{dm}^3$



It was shown that utilizing fixed media and limiting DO, more nitrite could be accumulated and therefore, conversion of nitrite to nitrate could be prevented. It would be a highly cost-effective process which requires less energy, oxygen and carbon source. Conventional processes of ammonium oxidation to nitrite, nitrite oxidation to nitrate, reduction of nitrate to nitrite and then conversion of nitrite to nitrogen gas (N_2) cost too much for wastewater treatment plants [20]. Table 3 shows ammonium removal efficiency, SND efficiency, SNR, SDNR and NAR in SBR and SBBR processes.

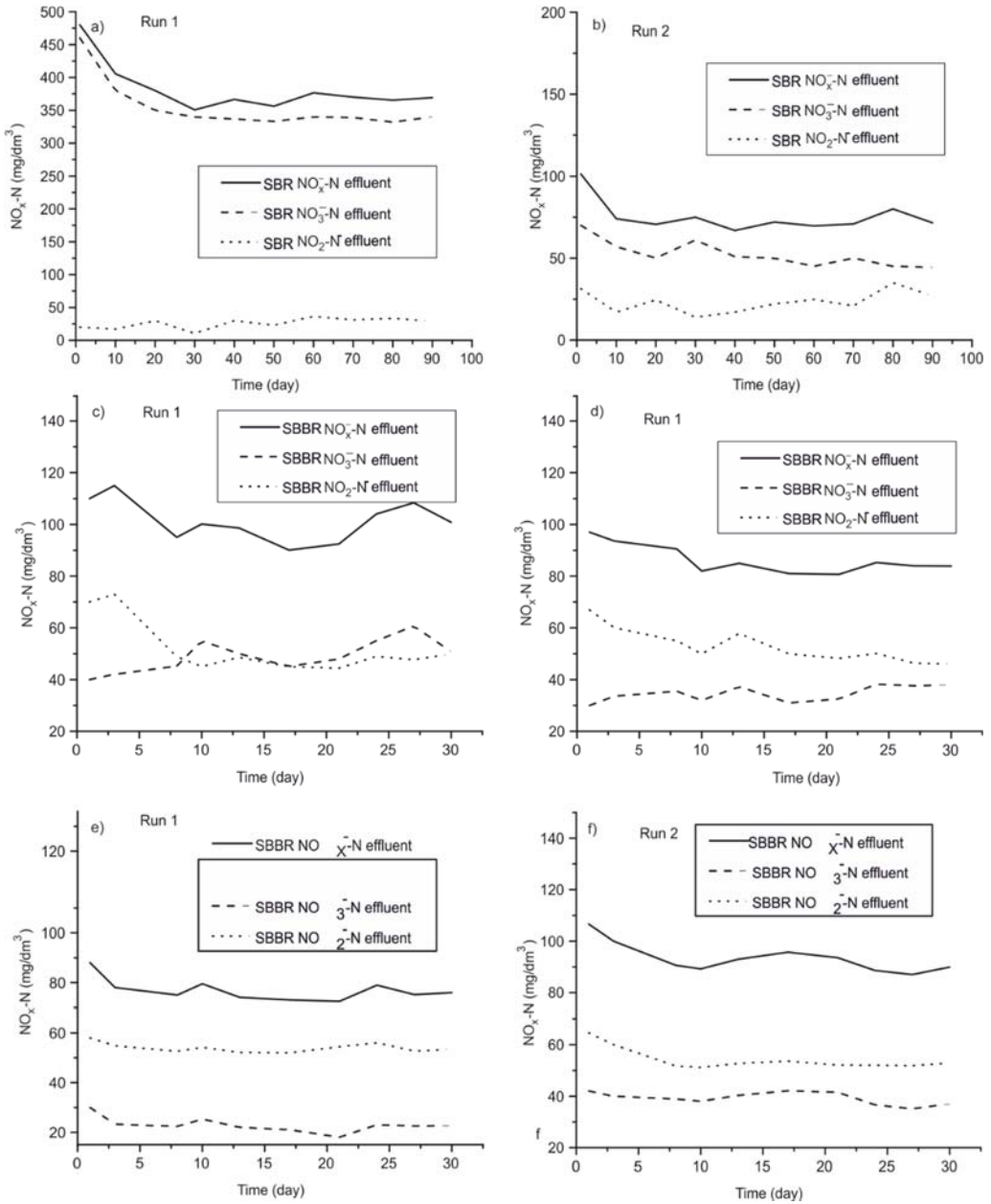


Fig. 7. Concentration of effluent $\text{NO}_x\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ in SBR and SBBR operation:

- a) SBR in run 1, b) SBR in run 2, c) SBBR in run 1 at the DO level of 1 mg O₂/dm³,
 d) SBBR in run 1 at the DO level of 0.5 mg O₂/dm³, e) SBBR in run 1 at the DO level of 0.3 mg O₂/dm³,
 f) SBBR in run 2 at decreased DO level from 1 to 0.5 mg O₂/dm³

Table 3

The results of the reactor operations at various steps of the study

Reactor ^a	Run	NLR [kg N/(m ³ ·d)]	OLR [kg COD/(m ³ ·d)]	Ammonium removal efficiency [%]	SNR ^b	SDNR ^c	SND efficiency [%]	NAR [%]
SBR	1	2.5	10	81	7.83	–	–	–
SBBR (a)				84.5	–	–	83.8	70.2
SBBR (b)				92	–	–	90.9	87
SBBR (c)				98.4	–	–	94.1	90.3
SBR	2			86.9	9.24	18.56	–	–
SBBR (d)				97.3	–	–	86	79.6

^aSBBR with fixed DO level of a) 1, b) 0.5, c) 0.3 mg O₂/dm³, and d) SBBR with decreasing DO level from 1 to 0.5 mg O₂/dm³ and then 0.3 mg O₂/dm³.

^bSNR in mg NH₄⁺-N/(g MLVSS·h).

^cSDNR^c in mg NO₃⁻-N/(g MLVSS·h).

3.3. EFFECT OF DECREASED DO IN RUN 2

SBR cycle involved an anoxic phase in run 2 between 10th hour and 12th hour of the cycle. Considering anoxic phase, it would reduce the effluent nitrate concentration of SBR. The aim of this modification was achieving the best result of SBR in nitrogen removal with respect to SBBR result. Internal carbon source was added at the beginning of the anoxic phase. TN removal efficiency of SBR reached 87.4% in run 2. Ammonium removal efficiency increased slightly, from 81% in run 1 to 86.9% in run 2. Nitrate concentration in SBR effluent was reduced by 289.35 mg/dm³ and reached 66.9 mg/dm³.

Figure 8a describes the evolution of nitrogen compounds during a typical cycle of SBR in run 2. Ammonium concentration decreased under aerobic phases and increased immediately after adding the internal carbon source. On the other hand, nitrate concentration increased under aerobic phases and decreased under anoxic phase due to dilution after addition of carbon source. Consecutive aerobic and anoxic phases increased SNR in run 2 in comparison to run 1 and it reached 9.24 mg NH₄⁺-N/(g MLVSS·h). SDNR was 18.56 mg NO₃⁻-N/(g MLVSS·h) in run 2 which means in average, 0.77 mg of nitrate was biodegraded by microorganisms during each hour of SBR cycle. SBBR operated at decreased DO concentration in run 2. Figure 8b shows a typical cycle of SBBR in run 2. DO was 1 mg O₂/dm³ at the beginning of the cycle until 8th hour then it was reduced to 0.5 mg O₂/dm³ between 8th hour and 16th hour and finally was reduced to 0.3 mg O₂/dm³ from 16th hour until the end of the cycle. The effluent nitrate concentration in SBBR was 69.5 mg/dm³ in run 2. SBBR ammonium removal efficiency was 97.3% in run 2 which was a bit less than the maximum

of efficiency in run 1 (98.4%). The reason of this slight decrease was that at a decreased DO, NOB needed less time for their activity but in fixed DO level the bacteria had no room to survive. In contrast of the best result of run 1 (at DO of $0.3 \text{ mg O}_2/\text{dm}^3$), SND efficiency decreased by 8.1% in run 2. NAR was decreased to 79.6% at decreased DO concentration in run 2.

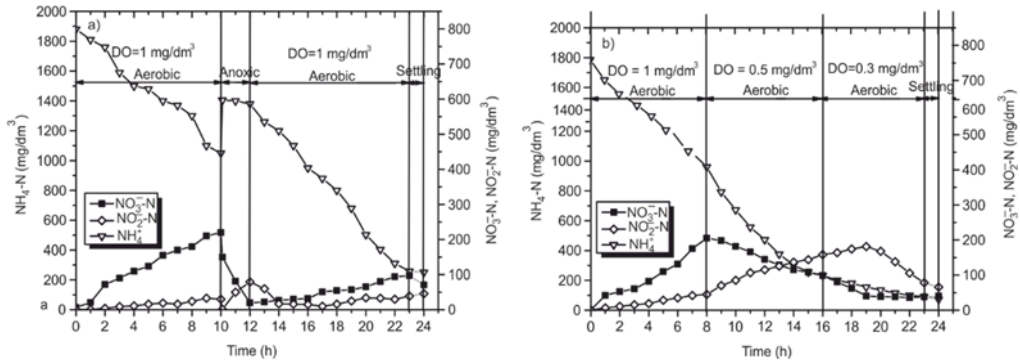


Fig. 8. Nitrogen compounds evolution during a typical cycle of SBR and SBRR in run 2:
a) SBR, b) SBRR

There are a number of approaches to increase NAR and to prevent conversion of nitrite to nitrate including: DO limitation, pH limitation, temperature fixing and free ammonia (FA) inhibition [21]. In this research, SND via nitrite which increased NAR, was achieved by DO fixing. Therefore, other factors did not affect the process under specific operation condition. Temperature was constant to prevent its effect on NAR. FA and pH have direct correlation with each other. Fixing pH leads to fixing FA, for example, at pH 7, FA is lower than 1% of total ammonia and at pH 11, FA is higher than 98% of total ammonia [22]. FA inhibits NOB by affecting its cell wall but the nitrogen compound does not kill the bacteria. A few authors reported that FA inhibits NOB and it has a little effect on AOB [23]. Temperature, pH and FA had no effect on SND via nitrite and the only factor which affected the activity of NOB was DO limitation. DO minimization increased NAR and improved SND via nitrite.

According to Fig. 8b, the slope of the ammonium profile versus time in first aerobic phase ($\text{DO} = 1 \text{ mg O}_2/\text{dm}^3$) was similar to that in the second aerobic phase ($\text{DO} = 0.5 \text{ mg O}_2/\text{dm}^3$). $\text{NO}_3\text{-N}$ concentration was decreased in second aerobic phase of SBRR in run 2 while it was increased in first aerobic phase. This happened because DO decrement in SBRR process could act similar to an anoxic phase so nitrate reduced more at fixed DO level of $0.5 \text{ mg O}_2/\text{dm}^3$. Nitrite contents slightly increased during the cycle and in contrast of run 1 (fixed DO level of $0.3 \text{ mg O}_2/\text{dm}^3$), reduction of nitrite concentration happened later at the third aerobic phase ($\text{DO} = 0.3 \text{ mg O}_2/\text{dm}^3$).

3.4. OXIDATION-REDUCTION POTENTIAL, pH AND DO PROFILES

ORP curve versus time can be utilized to detect the end of nitrate production and nitrate consumption. Under aerobic condition, ORP increases upon increasing time until an inflection point called α ORP which indicates the end point of nitrification. Under anoxic conditions, ORP versus time is down-trended until an inflection point so-called nitrate knee which indicates the end point of denitrification. Further, inflection points of ammonia valley and nitrate apex in pH profile versus time indicate the end point of nitrification and denitrification, respectively [2]. Also, extremum points of DO profile versus time show nitrification and denitrification characteristics points. Figure 9 presents ORP, pH and DO profiles during a typical cycle of SBR in run 2.

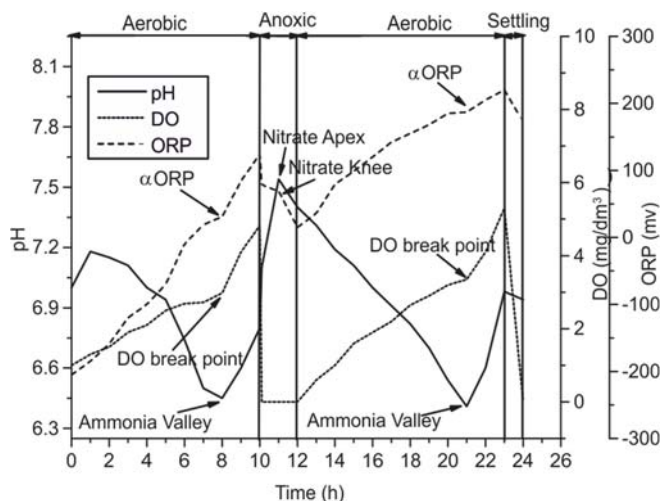


Fig. 9. ORP, pH and DO profiles of a typical cycle of SBR in run 2

At the 8th hour of the cycle (first aerobic phase) and 21th hour of the cycle (second aerobic phase) α ORP inflection points, DO break points as well as ammonia valley inflection points were observed, simultaneously. ORP exceeded 200 mV in the second aerobic phase while it was lower than 150 mV at the first aerobic phase (in 10 h). Moreover, the inflection points of nitrate knee and nitrate apex occurred simultaneously after 11 h from the start of the cycle under anoxic condition while pH reached 7.6. SBR cycle contained aerobic and anoxic phases in run 2 so the profile could contain all inflection points which described before. These indexes helped to ensure that complete nitrification and denitrification were happened without adding fixed film. Partial nitrification was achieved in SBBR, therefore, the inflection points could not be recognized. In Fig. 9 at the beginning, by introducing organic substrate, oxygen acted as electron acceptor. If influent DO was constant, inflection point of α O₂ or DO break point in DO profile indicates the end of nitrification. Organic matter oxidation at the beginning consumed too

much oxygen which led to constant DO level or slight increment of DO. When ammonium was almost depleted, DO was increased suddenly and DO break point was recognized. DO break point was recognized at 8th hour of the SBR cycle because more DO demand resulted in more oxygen consumption than SBBR.

3.5. AMMONIUM REMOVAL EFFICIENCY

Ammonium removal efficiency was evaluated in 150 days of long term study of the proposed processes divided to periods a, b, c and d. Periods a–c lasted for 30 days while period d lasted 60 days. The operational conditions of SBR during the periods a–c similar (DO level of 1 mg/dm³ and no anoxic phase). The operational conditions of SBBR during periods a (DO concentration of 1 mg O₂/dm³), b (DO concentration of 0.5 mg O₂/dm³) and c (DO concentration of 1 mg O₂/dm³) were similar to the conditions of run 1. The operational conditions during period d were similar to those during run 2 in which SBR cycle contained an anoxic phase and SBBR was operated at decreased DO level (8 h at the DO level of 1 mg O₂/dm³, 8 h at the DO level of 0.5 mg O₂/dm³ and 8 h at the DO level of 0.3 mg O₂/dm³).

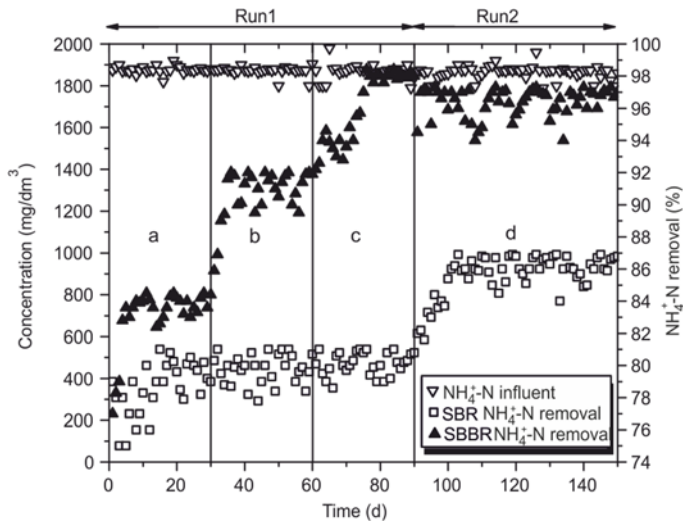


Fig. 10. Ammonium removal efficiency: a) run 1 SBR and SBBR at the DO level of 1 mg O₂/dm³, b) run 1 SBR at the DO level of 1 mg O₂/dm³ and SBBR at the DO level of 0.5 mg O₂/dm³, c) run 1 SBR at the DO level of 1 mg O₂/dm³ and SBBR at the DO level of 0.3 mg O₂/dm³, d) run 2 SBR with 2 aerobic phases at the DO level of 1 mg O₂/dm³ and an anoxic phase, and SBBR at decreased DO from 1 to 0.5 mg O₂/dm³ and then to 0.3 mg O₂/dm³

Figure 10 shows ammonium removal efficiencies of SBR and SBBR during a long term study. As can be seen, ammonium removal efficiency of SBBR was generally higher than that of SBR. The biofilm carrier effective surface area of 369.6 m²/m³ made

it easy to remove more ammonium from high strength ammonium wastewater. Small ammonium molecules diffused within the formed nitrifying biofilm and a surface biochemical reaction occurred. Adding an internal carbon source instead of an external one at the adjusted C/N ratio of 4 made it possible to survive for both nitrifying bacteria (autotrophic) and carbon oxidizing bacteria (heterotrophic) even at high level of soluble organic matters. Nonetheless, fixed biofilm enabled simultaneous conversion of accumulated nitrite to nitrogen. Influent ammonium concentration (1875 mg/dm^3) decreased versus time in SBBR cycles of period a (310.5 mg/dm^3 at the end of the cycle), period b (155 mg/dm^3 at the end of the cycle), period c (46 mg/dm^3 at the end of the cycle) as well as period d (70.6 mg/dm^3 at the end of the cycle) but the rate of ammonium conversion was higher at the DO level of $0.3 \text{ mg O}_2/\text{dm}^3$. Fixed biofilm carriers provided more mass transfer in comparison of moving biofilm carriers or suspended growth systems. In other systems, the turbulence of the bulk liquid could cause lower ammonium removal efficiency at a similar DO concentration but in the present study ammonium removal efficiency was almost higher than 90% during all SBBR operations.

Table 4

SBR performance according to various authors

Ammonium removal [%]	Reactor	External carbon source	C/N	Oxygen conditions	DO level during aerobic phase [$\text{mg O}_2/\text{dm}^3$]	SRT [d]	SNR ^a	SDNR ^b	Reference
50	SBR	no	–	aerobic	3	5	42±2	–	[8]
50	SHARON				3	1	39±2		
99	activated sludge				2	53	20.41		
14.89 ^c	SBR	yes	2.74	aerobic	1.0–1.5	15	14.43	53.11	[25]
86.9	SBR	no	4	/anoxic	1	25	9.24	18.56	present study

^aSNR in $\text{mg NH}_4^+\text{-N}/(\text{g MLVSS}\cdot\text{h})$.

^bSDNR in $\text{mg NO}_3^-\text{-N}/(\text{g MLVSS}\cdot\text{h})$.

^cNitrification ratio as ammonium removal ($\text{mg NH}_4^+\text{-N}/(\text{dm}^3\cdot\text{h})$).

Table 5

Table 5. SBBR performance according to various authors

Ammonium removal [%]	Reactor	pH	NLR [$\text{kg N}/(\text{m}^3\cdot\text{d})$]	DO level [$\text{mg O}_2/\text{dm}^3$]	HRT [h]	SRT [d]	Process	Reference
98	SBBR	7.6	0.9	0.5	96	–	CANON	[26]
80		8±0.2	160 ^a	0.8–1.0	48	–	CANON	[27]
58.2		7–7.6	400 ^a	<2	24	16	partial nitrification	[9]
98.4		7	2.5	0.3	24	55	SND via nitrite	present study

^aInfluent ammonium concentration ($\text{mg NH}_4^+\text{-N}/\text{dm}^3$) instead of NLR.

SBBR ammonium removal efficiency reached 98.4% in days 76 and 84 (period c). The efficiency was 92% in days 36, 41, 46, 53 and 59 (period b), and 84.5% in days 11, 19 and 28 (period a). In Tables 4, 5, the results of the present study have been compared with those of other authors. Ammonium removal efficiency of SBBR was higher in period c (run 1 at fixed DO concentration of 0.3 mg O₂/dm³). These results can be explained by providing adequate depth of penetration for oxygen molecules within the biofilm as well as providing enough time for nitrification and denitrification. Removal efficiency was not stable in period d at decreased DO concentration but it was higher than 94% during most time of this period.

3.6. STATISTICAL ANALYSIS

Statistical analysis was done and significance of differences between the results was studied while each test was repeated 4 times. First, normality of data was investigated by using the Kolmogorov–Smirnov test. The data distribution of the results of N removal in SBR and SBBR processes were normal (for example, the significance was higher than 0.05 for SBR) (Tables 6 and 7).

Table 6

Results of the Kolmogorov–Smirnov test for N removal in SBR

Number of parameters, <i>N</i>		8
Normal parameters ^a	Mean	83.9500
	Std. deviation	3.23198
Most extreme differences	Absolute	0.276
	Positive	0.276
	Negative	-0.227
Test statistic		0.276
Asymptotic significance (2-tailed)		0.074 ^b

^aTest distribution is normal, calculated from data.

^bLilliefors' significance correction.

Table 7

Results of the Kolmogorov–Smirnov test for N removal in SBBR

Number of parameters, <i>N</i>		16
Normal parameters ^a	Mean	93.0813
	Std. deviation	5.70102
Most extreme differences	Absolute	0.220
	Positive	0.146
	Negative	-0.220
Test statistic		0.220
Asymptotic significance (2-tailed)		0.038 ^b

^aTest distribution is normal, calculated from data.

^bLilliefors' significance correction.

Then, one way analysis of variance ANOVA was done to study the significance of data. The ratio of variances F was calculated and F -distribution table was utilized to obtain critical F (F_{crit}). The significance level of 0.005 was assumed. If F was higher than F_{crit} or the significance level was lower than 0.005, the results were evaluated significantly different. F_{crit} of SBR and SBBR operations using degrees of freedom (df) was 18.63 and 7.23, respectively. According to Tables 8 and 9, the results were significantly different.

Table 8

ANOVA test for N removal in SBR

	Sum of squares	df	Mean square	F	Significance
Between groups	69.620	1	69.620	119.349	0.000
Within groups	3.500	6	0.583		
Total	73.120	7			

Table 9

ANOVA test for N removal in SBBR

	Sum of squares	df	Mean square	F	Significance
Between groups	478.027	3	159.342	201.327	0.000
Within groups	9.498	12	0.791		
Total	487.524	15			

4. CONCLUSION

SBR and SBBR were utilized to treat simulated ammonium-rich pharmaceutical wastewater which had high COD. SBR nitrogen removal efficiency with anoxic phase was higher than SBR without anoxic phase. When decreased DO concentration strategy during a cycle was carried out, SBBR ammonium removal efficiency was higher than that of SBR. SBR effluent nitrate concentration was a little lower than that of SBBR with decreased DO concentration. Although, when SBBR operated at fixed DO concentration, the effluent nitrate level was minimum. The nitrite accumulation rate (NAR) increment and DO decrement had high correlation. It was summarized that upgrading SBR to SBBR through adding fixed bed and DO adjustment improves the efficiency of removal of nitrogen compounds. SBBR micro-zones provide simultaneous anoxic and aerobic conditions, and nitrification and denitrification (SND) as well. Nitrite is obtained in a single reactor. SBBR process at high nitrogen loading rate (NLR) and low DO concentration has acceptable nitrogen removal efficiency during long time operation.

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