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Simultaneous nitrification-denitrification in a sequencing batch reactor equipped with fixed Kaldnes carriers

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Original Article

Abstract

The main aim of this study was to evaluate the performance of a modified sequencing batch reactor (MSBR) using fixed Kaldnes carriers fed with acclimated sludge for ammonium removal via simultaneous nitrification-denitrification (SND) in synthetic wastewater. The results exhibited a SND of 82.3% within a 450-minute cycle time which was higher than that of a SBR without carrier (69.83%). Nitrite accumulation rate (NAR) increased from 16.94% to 32.83% until 120 minutes of cycle time, and then, decreased to 1.17% by 450 minutes. The biomass concentration in the bio-film (674 ± 6 mg/l) was lower than suspended biomass (1984 ± 12 mg/l). However, the specific oxygen uptake rate (SOUR) of the bio-film (5.24 ± 0.28 mg O_2/mg MLVSS.d) was greater than suspended biomass (1.89 ± 0.12 mg O_2/mg VSS.d), indicating the higher bioactivity of the bio-film than that of suspended biomass. Up to 3% salinity had no significant effect on MSBR performance for both chemical oxygen demand (COD) and ammonium removal. These results illustrated the high efficiency of the MSBR in the treatment of wastewater containing high salinity as well as the removal of nitrogen compounds via SND.

KEYWORDS: Nitrification, Denitrification, Salinity, Wastewater, Biomass

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Introduction

In recent years, nitrate contamination of water resources due to discharge of domestic and industrial wastewater and extensive use of nitrogenous fertilizers has become a serious environmental concern.[1] The increasing concentration of nitrate in aquatic ecosystems can cause eutrophication (algal bloom).[2] In addition, high levels of nitrate in drinking water cause serious health problems such as methemoglobinemia in infants and gastric cancer.[3] Several methods, such as the reverse osmosis,[4] ion exchange,[5] electrochemical and bio-electrochemical processes,[1,6] adsorption,[7] electrocatalytic reduction,[8] and biological processes,[9] have been used for the removal of nitrate from aquatic environment. Biological nitrification-denitrification is the most commonly used process for nitrogen removal from wastewater.[10] However, it is more economical to combine nitrification and denitrification via a simultaneous nitrification-denitrification (SND) process in the same reactor than it is to perform the two processes separately.[11-14] Recently, SND process has been described for various wastewater treatment systems. These processes are used because they do not require an anoxic tank and they allow the pH to be maintained at a neutral level without the need for pH adjustment with an external acid-base

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0source.[14,15] Sequencing batch reactor (SBR) has been successfully used in wastewater treatment for chemical oxygen demand (COD) and nitrogen removal as an alternative to a conventional activated sludge system due to its advantages, including flexibility in operation, simplicity in structure, and the ability to meet many different treatment objectives.[16,17] This reactor has been successfully applied for SND process and even biological phosporous removal (BPR).[18-20] However, SBRs have a number of problems, such as low ability to settle sludge, high excess sludge production under high organic loading rates, and low removal efficiency due to their limitation in biomass production.[2,16,21,22] To solve these problems, different carriers have been used in the SBR in fixed or fluidized forms to increase the biomass concentration, and to overcome difficulties with respect to the maintenance of microorganisms and the slow growth of microorganisms. The support material is an important factor that keeps the microorganisms in the reactor by means of bio-film growth.[23] Over the past few years, researchers have studied municipal wastewater treatment using a bio-film SBR with different carriers.[22-25]

As a new alternative, in the present study, the Kaldnes carrier was applied for SND in the SBR. However, the application of this carrier for some biological systems such as SBR is limited due to its buoyancy. Hence, the Kaldnes carrier was packed, and then, placed in the SBR as a modified SBR (MSBR) to increase the micro-anoxic and micro-aerobic zone in the reactor for the enhancement of SND performance. To the best of our knowledge and based on our literature review, there is no report on the application of Kaldnes carrier as fixed-bed in the SBR for SND, together with the removal of COD in high saline wastewater treatment.

Materials and Methods

The experimental reactor was a rectangular tank made of Plexiglas with a working volume of 10 l and a free board of 5 cm. A flow diagram of the experimental reactor is shown in figure 1.

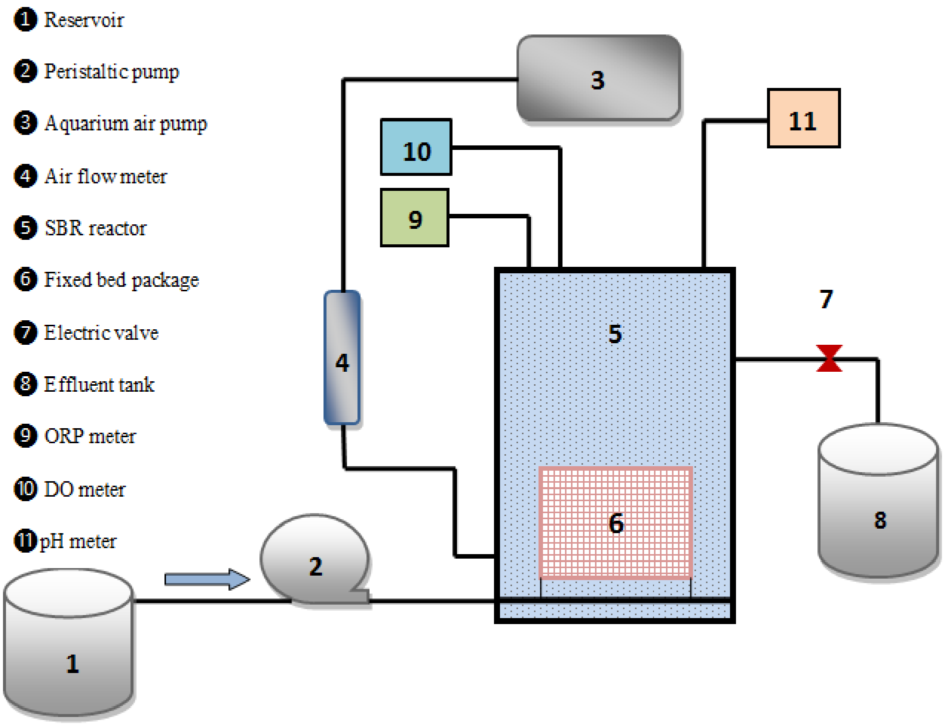

Figure 1. Schematic flow diagram of the experimental reactor

The reactor was provided with a peristaltic pump (Etatron, Italy), an air pump, an air flow meter (TG, Italy), and an electric valve. The MSBR reactor was filled with Kaldnes carriers (30% of the total volume of the MSBR) for growth of the bio-film. The length, diameter, density, and surface area of each carrier were 7 mm, 10 mm, 170 kg/m^3, and 24 m^2, respectively. The MSBR operation includes the four phases of filling (120 minutes), aeration (300 minutes), settling (30 minutes), and decanting (30 minutes), with a total time of 8 hours and 3 cycles in each day. The peristaltic pump was set to the flow rate of 1.5 l/hour to feed the wastewater into the reactor within a filling time of 120 minutes. The total volume of 3 l was exchanged in each cycle. Hydraulic retention time (HRT) was defined as the working volume of the SBR reactor divided by the total volume of synthetic wastewater fed into the reactor during one operational day.[2] Accordingly, a fixed HRT of 1600 minutes was attained on the basis of the total volume of 9 l fed into the reactor during one operational day and the reactor working volume of 10 l. These calculations are in accordance with the report of Wang et al.[20] The reactor was equipped with a portable dissolved oxygen (DO) meter (Jenway 9200, UK) and a portable oxidation-reduction potential (ORP) meter (ORP Tester 10, Malaysia). The timing of each phase (filling, aeration, settling, and decanting) of the reactor was controlled with adjustable timers. Moreover, using a portable pH meter (pH Tester 10, Malaysia), the pH of the system was maintained between 7.0 and 8.0.

The reactor was inoculated with activated sludge taken from return activated sludge line of a municipal wastewater treatment plant in Tehran, Iran. The reactor start-up involved daily feeding of synthetic wastewater until a biofilm started to form on the surface of carriers after 8 weeks. During this period, the reactor operated in a "fill and draw" mode for the formation of a bio-film layer on the Kaldnes surface. The COD:N:P ratio in the wastewater during the acclimation period was kept at 100:5:1. In the "fill and draw" mode, ammonium, COD, mixed liquid suspended solid (MLSS), and sludge volume index (SVI) were measured daily. After measurement, the air pump was switched off and the sludge was allowed to settle at the bottom of the reactor. Then, 10% of the volume of the reactor was withdrawn and replaced with synthetic wastewater consisting of $MgSO_4.7H_2O$ (69.6 mg/l), $FeCl_2{\cdot}4H_2O$ (17.25 mg/l), $CaCl_2{\cdot}2H_2O$ (22.5 mg/l), $CuSO_4{\cdot}H_2O$ (0.08 mg/l), $Na_2MoO_4{\cdot}2H_2O$ (0.15 mg/l), $MnSO_4{\cdot}H_2O$ (0.13 mg/l), $ZnCl_2$ (0.23 mg/l), and $CoCl_2{\cdot}6H_2O$ (0.42 mg/l).[26] The ammonium and COD concentrations reached 25 mg NH_4-N/l and 500 mg/l, respectively, and aeration was resumed. After the acclimation period, the MLSS and SVI reached approximately 2000 mg/l and 125 ml/g, respectively. Once the system reached steady state conditions and a suitable bio-film had grown on the carriers, the reactors were operated in series in a cyclic test mode. Ethanol (C_2H_5OH), ammonium chloride (NH_4Cl), and phosphate hydrogen potassium (HK_2PO_4) were used as carbon, nitrogen, and phosphorus sources, respectively. The C:N:Pratio in the simulated wastewater was similar to that in the "fill and draw" mode.

COD, MLSS, volatile MLSS (MLVSS), and SVI were determined according to standard methods for the examination of water and wastewater.[27] SVI was calculated as ml/g by dividing the results of the settling test (ml) by the MLSS concentration in the reactor. Samples were filtered with 0.45 µm filters, and then, NH_4-N, NO_3-N, and NO_2-N contents were colorimetrically measured using standard methods with a UV-Vis spectrophotometer (Unico 2100) at 425, 220, and 543 nm, respectively. In the case of NO_3-N measurement, the interference of the organic matter was eliminated using the following equation:

$$A_{corr} = A_{220} - 2 \times A_{275} \qquad (1)$$

where A_{corr}, A_{220}, and A_{275} are the corrected UV-light absorbance of nitrate, absorbance at 220 nm, and absorbance at 275 nm, respectively.[28] Collected samples were acidified by adding 0.2 ml H_2SO_4 in a concentrated form to 100 ml of each sample and stored at 4 °C for analysis on the next day.[29] A scanning electron microscope (SEM) (Philips XL 30, the Netherlands) was applied to study the morphological details on the bio-film surface. DO, pH, and ORP were measured using specific probes.

To estimate the amount of bio-film attached to the surface of each carrier, 20 carriers were selected randomly from the reactor. The carriers were separated from the reactor and dried until constant weight was achieved. The dried carriers were weighed to calculate the total mass (M_t) of the carriers with bio-film. Then, the net weight of the carriers without bio-film (M_c) was estimated after washing and cleaning the carriers. After that, the amount of bio-film attached to the surface of 20 carriers (BS_{20}) was calculated according to equation 2.

$$BS_{20} = M_t - M_c \quad (2)$$

According to the total number of carriers in the package of the reactor (861), the total amount of bio-film in the reactor (BS) can be estimated using equation 3.[25]

$$BS = BS_{20} \times \frac{861L^{-1}}{20} \quad (3)$$

The specific oxygen uptake rate (SOUR) was used to evaluate the bioactivity of the bio-film attached to the surface of the Kaldnes carriers. The SOUR test was performed in Erlenmeyer flasks equipped with DO meters. Randomly, 10 carriers and 100 ml of mixed liquid suspended solid were removed from the reactor and stored in two separate flasks for comparison. First, the Erlenmeyer flasks were aerated until an initial concentration of about 8 mg O_2/l was achieved. Then, the reduction in the oxygen content was monitored and recorded via DO meter. The analysis was terminated when the DO concentration decreased to about 1 mg/l. Ethanol was used as the carbon source and was added at a 200 mg/l COD concentration to each flask. Finally, the SOUR was calculated by dividing the oxygen uptake rate (OUR) by the MLVSS concentration.[30,31]

Results and Discussion

Acclimation period and bio-film growth

Biomass acclimation has been a key factor for improving nitrification performance in biological reactors.[32] In this study, acclimation was carried out for 30 days with 500 mg/l COD and 25 mg NH_4-N/l. The variations in SVI and MLSS versus the effluent COD and NH_4-N during the 30 days of the acclimation period are shown in figure 2. During this period, effluent COD and NH_4-N concentrations reached 5.57 and 1.05 mg/l, respectively. As can be seen in figure 2, the decrease in COD and NH_4-N concentrations was achieved through the reduction of MLSS concentration from 6500 to 1950 mg/l, while the SVI increased from 51 to 123 ml/g.

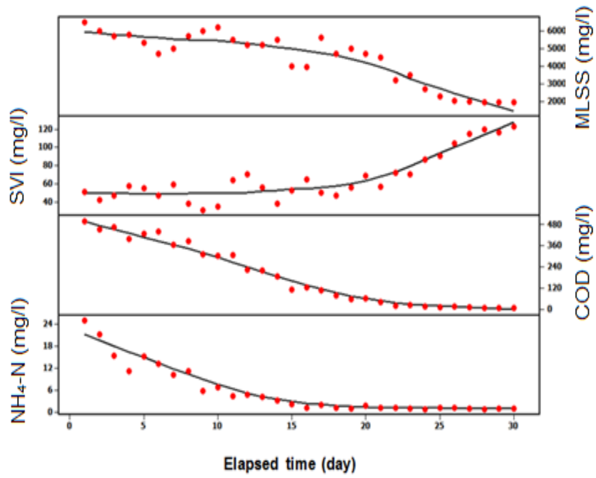

Figure 2. Variations in chemical oxygen demand (COD) and ammonium versus variations in sludge volume index (SVI) and mixed liquid suspended solid (MLSS) during acclimation period (Initial COD concentration = 500 mg/l, initial ammonium concentration = 25 mg NH_4-N/l)

Surface Morphology of the attached bio-film onto the carrier

Figure 3 [(a-1) and (a-2)] shows the structure of the upper and lower surface of the Kaldnes carrier without bio-film at 5000X magnification and displays a smooth carrier surface for bio-film growth. The SEM image in figure 3 shows the fully grown bio-film on the surface of the carriers. Moreover, according to figure 3 (b-1), the presence of bacteria as attached bio-film is evident, thus indicating that an appropriate environment for the removal of pollutants by microorganisms has been achieved. This structure can also create many suitable anoxic and aerobic micro-zones to enhance SND processes in the same reactor. According to figure 3 (b-2), after the experiments related to salinity, the bio-film structure was not considerably different from the structure shown in figure 3 (b-1), implying the resistance of bio-film against undesirable conditions such as salinity.

Bio-film growth

The concentration of biomass attached onto the surface of the Kaldnes carrier was calculated according to equations (3) and (4). Moreover, the amount of suspended biomass in the reactor was calculated according to the method described in standard methods for the examination of water and wastewater.[27] The biomass concentration of the bio-film and the suspended biomass in the reactor were 674 ± 5 and 1984 ± 12 mg/l, respectively. The biomass concentration of the bio-film attached onto the surface of the media was lower than that of suspended biomass. Nevertheless, the ammonium removal efficiency of the SBR with the carrier was higher than that of the SBR. This finding shows that the bio-film had higher bioactivity than the suspended biomass.

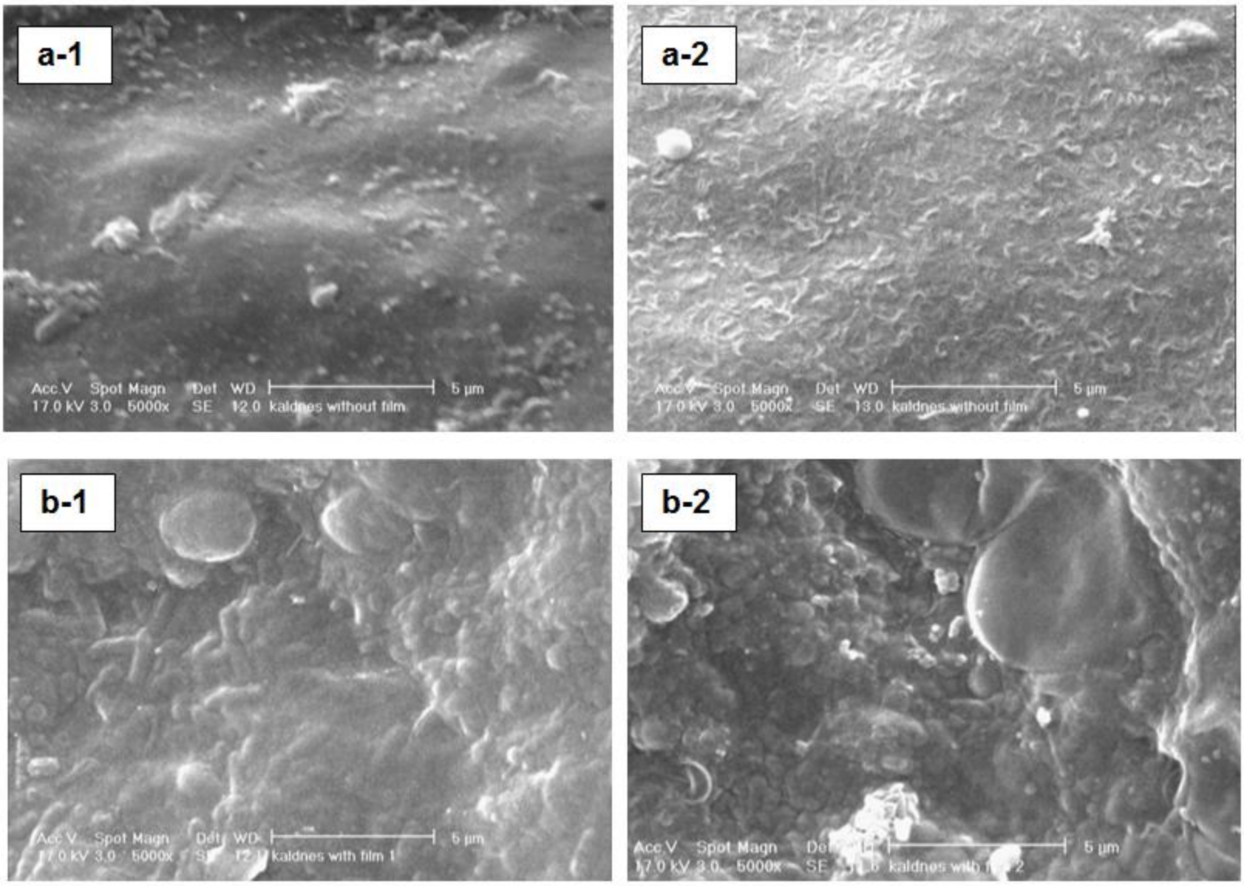

Figure 3. Scanning electron microscopy (SEM) images of the (a-1) upper and (a-2) lower surface of the carrier without bio-film, (b-1) the carrier with bio-film and (2-b) the surface morphology of the bio-film after ammonium and chemical oxygen demand (COD) removal in saline wastewater taken at 5000 X magnification

Specific oxygen uptake rate (SOUR)

The SOURs of the bio-film and the suspended biomass were 5.24 ± 0.28 and 1.89 ± 0.12 mg O_2/mg MLVSS.d, respectively. The SOUR for the attached and suspended biomass showed that the respirations and catabolic activities of the microorganisms in the bio-film were higher than those of the suspended biomass. This indicates the high bioactivity of the microorganisms attached onto the Kaldnes surfaces. Chen et al. showed that the granules in a SBR reactor have better bioactivity in terms of SOUR than the suspended sludge.[33]

Simultaneous nitrification-denitrification process in MSBR

To treat synthetic wastewater that contains ethanol as the carbon source, the evaluation of the removal of NH_4-N and NO_3-N were carried out using a SND process during an operational cycle in a MSBR reactor. Combining nitrification and denitrification for complete ammonium removal in the same tank is an efficient way to reduce the operational requirement related to separate processes in treating wastewater.[34] Variations of NH_4-N, NO_3-N, and NO_2-N in cycle time are depicted in figure 4. As shown in figure 4, increased NO_3-N concentration during the aeration phase and then decrease in the produced NO_3-N at the end of the settling phase confirms the transformation of NH_4-N to N_2 gas. Furthermore, as seen at the end of the settling phase, NH_4-N and NO_2-N concentrations reached the lowest values, indicating N_2 gas production as a result of SND. This fact was also demonstrated in the investigation by Rodriguez et al.[19] At the beginning of the settling phase, the concentration of nitrate decreased because of the absence of aeration at a lower oxidation reduction potential (ORP). ORP is commonly studied for controlling and monitoring SND process because of the electromotive force developed when oxidizers or reducers are present in wastewater.[35] Favorable denitrification usually occurs along with decreasing ORP. ORP increases during aeration at high DO concentration, and then, decreases during the anoxic phase.[36]

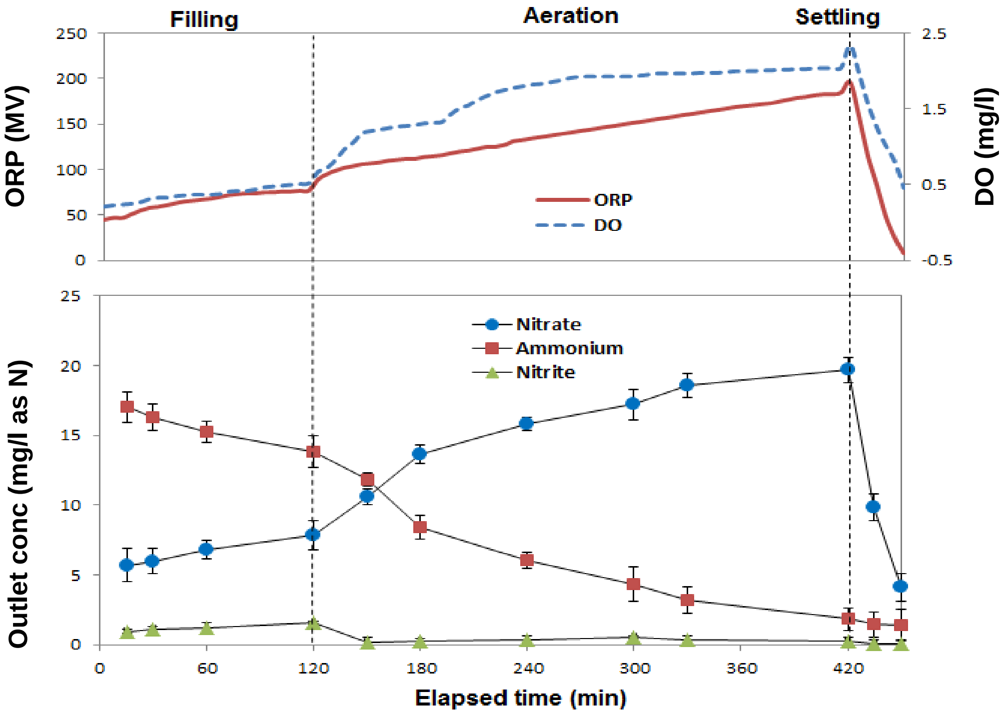

Figure 4. Variations in the nitrogen species concentration, and dissolved oxygen (DO) and oxidation-reduction potential (ORP) values versus elapsed time (Initial ammonium concentration = 25 mg NH_4-N/l)

Figure 4 shows the profile of DO and ORP during the SND to evaluate the effect of oxidative conditions in the reactor for simultaneous removal. As shown in figure 4, the greatest ORP decline zone occurred at the end of the settling phase when the dissolved oxygen reached its lowest value. At this point, the DO and ORP values were 0.46 mg/l and 8 mV, respectively. Moreover, figure 4 shows that the highest ORP that occurred at approximately the end of the aeration phase with a value of 196 mV matched the high DO concentration (2.34 mg/l). Thus, we can use ORP variations in the system as an efficient means for evaluation of oxidative and reductive conditions. Comparatively, a DO concentration between 2.5 and 4.0 mg/l was recommended for SND in a study conducted by Li et al.[11] They used an aerobic sequencing batch biofilm reactor (SBBR) packed with Bauer rings, which had higher energy-consumption than the present work. Subsequently, by using MSBR, they evaluated the feasibility of SND.[11] The following equation was used to calculate the efficiency of SND in the MSBR reactor.[18]

$$\text{Efficiency}_{\text{SND}}(\%) = \left(1 - \frac{\text{NO}^-_{\text{x remained}}}{\text{NH}^+_{4\text{ oxidized}}}\right) \times 100 \qquad (4)$$

where $\text{NO}^-_{\text{x remained}}$ is the remaining NO_3-N/NO_2-N and $NH^+_{4\,oxidized}$ is the oxidized NH_4-N after the reaction. As seen in figure 4, by increasing reaction time to 450 minutes (at the end of the settling phase), the effluent concentrations of NH_4-N, NO_3-N, and NO_2-N reached 1.43, 4.12, and 0.05 mg/l N, respectively. It seems that using a fixed bed Kaldnes carrier is appropriate for SND in a MSBR reactor. In the SBR reactor equipped with the Kaldnes carriers, the biofilm becomes thicker, the SND efficiency improves, and the effluent concentration is maintained at a low level.[37] It has been demonstrated that reduction in SND efficiency in conventional SBR with suspended biomass can be attributed to lower anoxic microzones within the flocs which are maximized in the bio-film attached onto Kaldnes carriers.[38] This statement was in agreement with the findings of Li et al., who found that thicker bio-film is beneficial for SND.[11] We know that ticker bio-film results in increased anoxic microzones within the film. It has been found that high concentrations of nitrite have a negative effect on the removal of ammonium via SND.[13] Therefore, to evaluate the performance of the reactor for ammonium oxidation, the nitrite accumulation rate (NAR) was estimated using equation 5.[15]

$$\text{NAR}(\%) = \frac{\text{NO}_2\text{–N}}{\text{NO}_2\text{–N} + \text{NO}_3\text{–N}} \times 100 \qquad (5)$$

Accordingly, the variations of NAR and nitrite are depicted in figure 5. Figure 5 shows a low NAR% during SND. NAR increased from 16.94% to 32.83% (equal to increasing nitrite from 0.96 to 1.58 mg NO_2-N/l) as the reaction time increased from beginning to 120 minutes. Then, NAR decreased from 32.83% to 1.17% (equal to decreasing nitrite from 1.58 to 0.05 mg NO_2-N/l) as the time increased from 120 minutes to 450 minutes. It was observed in another investigation that ammonium can be converted to N_2 gas without the accumulation of nitrite by the mixed culture during SND.[34] It has been confirmed that nitrite can be completely converted in the presence of sufficient carbon source during the SND. Zhang et al., in their study, observed that nitrite conversion increased from 27.59% at C/N of 5 to 100% at C/N of 20.[13] Our operational conditions regarding C/N ratio were consisted with the report of Zhang et al.[13] Therefore, sufficient C/N ratio, which was applied in the present work, was beneficial in terms of low accumulation of nitrite in the reactor during the SND. At the end of the experiments, the package of carriers was removed from the reactor and the SND efficiency was measured. This enabled the evaluation of the effects of the Kaldnes carrier on SND efficiency. At the end of the settling phase, the effluent concentrations of NH_4-N, NO_3-N, and NO_2-N reached 4.65, 6.05 and 0.09 mg/l N, respectively. According to equation 4, SND using SBR without Kaldnes carriers would be 69.83% which is lower than that of MSBR. This demonstrates that the capability of the MSBR for SND is higher than the SBR without the carrier.

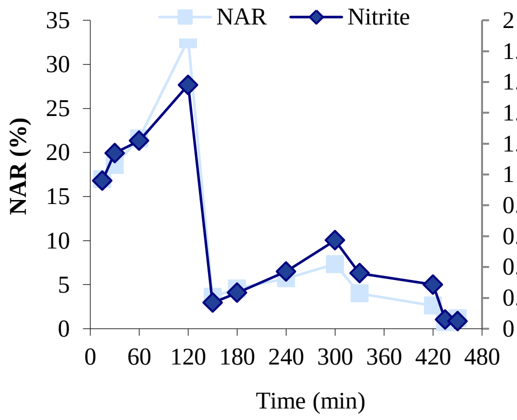

Figure 5. Variations in the effluent concentration of NO_2-N and nitrite accumulation rate (NAR) (%) versus reaction time

Effect of salinity on SND

High saline wastewater induces salt stress in the microbial communities in biological wastewater systems. Salinity can cause inhibition for many enzymes, inactivation of bacteria, and plasmolysis.[29] For example, metal refinery wastewater has high salinity which is difficult to treat biologically.[39] In this set of experiments, ammonium removal from saline wastewaters was investigated in MSBR over 62 days because of the negative effect of salinity on nitrification compared to denitrification. For example, in a study related to denitrification at high salinity, a high denitrification rate was achieved at 0%–10% NaCl.[39] The effects of salt concentration (0.5%–5% NaCl) on ammonium and COD removal efficiencies in MSBR were investigated. To evaluate the salinity effect, the MSBR cycle was fixed at 8 hours, as stated in the materials and methods section. To compare SBR with and without the media, ammonium and COD were adjusted to 50 mg NH_4-N/l and 1000 mg/l, respectively. As shown in figure 6, increasing the salinity from 0.5 to 5% led to the decrease of the removal percentage of ammonium and COD from 70.45% to 15.1% and from 99.05% to 28.12%, respectively. However, the COD removal percentage was higher than 90% for all tested salinity concentrations up to 1%.

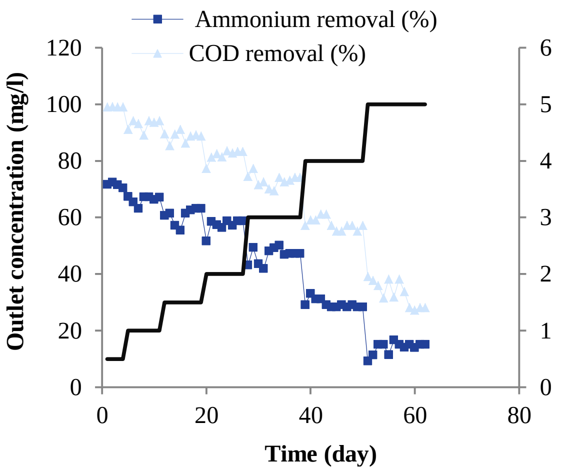

Figure 6. Effect of salinity on the removal percentage of ammonium and chemical oxygen demand (COD) during 62 days of operation (Initial COD concentration = 1000 mg/l, initial ammonium concentration = 50 mg NH_4-N/l)

With the increasing of salinity up to 3%, a significant decrease was observed in ammonium and COD removal percentages. At 4% salinity, COD and ammonium removal percentages reached 57.12 and 29.11%, respectively. Figure 6 shows the tolerance of the MSBR to salinity, up to 3%, for treating synthetic wastewater containing 50 mg NH_4-N/l and 1000 mg COD/l. The obtained results show that MSBR is suitable for treating wastewater containing high salinity. These results indicate that a sufficient acclimation period (62 days) and suitable growth of bio-film on the Kaldnes carriers can help overcome the stressing and inhibitory conditions related to high salinity in synthetic wastewater.[40] Biomass retention within a reactor by means of a carrier is a key factor in the prevention of washout of slow-growing nitrification bacteria, which can be more resistant to inhibitory conditions, such as salinity. Results obtained in this study were consistent with those attained by Bassin et al.[32] Their study showed a high nitrification percentage for municipal wastewater with salinity concentrations of up to 8000 mg/l, equal to 0.8% by means of SBR containing a carrier. Rene et al. performed a study on treating fish market wastewater by SBR and showed that increasing the salt concentration to 3% lowered the nitrogen removal percentage by 35% to 45%.[29] This effect was attributed to salt induced forces. After the evaluation of the effect of salinity on ammonium and COD removal in synthetic wastewater in a MSBR reactor, a sample of carrier was withdrawn and analyzed through SEM to determine the effect of salinity on bio-film. As shown in figure 3 (b-2), after the experiments related to salinity, the bio-film structure was not considerably different from the structure shown in figure 3 (b-1). However, other researchers have reported that salinity may potentially disturb bio-film compositions, such as extracellular polymeric substances, and can affect the oxygen transfer rate.[29]

Conclusion

This investigation confirmed the suitability of Kaldnes as a polyethylene carrier in a MSBR for a SND process. A close relationship was observed between decreasing ORP and nitrate reduction during SND in MSBR. SND results indicated that the SND efficiency of the MSBR was higher than that of SBR without Kaldnes carriers. The biomass concentration of the bio-film attached onto the carrier was lower than that of suspended biomass, but the higher SOUR of the attached biomass compare to the suspended biomass concentration indicated a higher bioactivity than the suspended biomass. Salinity had a low effect on decreasing the removal percentage, which ranged between 1% to 3% salinity. Finally, it should be stated that the application of SBR with Kaldnes carriers can be an effective way to treat wastewater containing high salinity and COD levels as well as to remove nitrogen compounds via SND.

Conflict of Interests

Authors have no conflict of interests.

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