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Effects of different concentration of Zn(II) on the organic material degradation and nitrification process in the treatment of real industrial wastewater

Farklı Zn (II) konsantrasyonlarının gerçek endüstriyel atıksuların arıtılmasında organik madde bozunması ve nitrifikasyon prosesi üzerine etkisi

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Abstract

In this study, three real industrial wastewaters containing different concentration of Zn (II) were treated with lab-scale sequential batch bioreactor (SBR) to determine the effects of Zn (II) on the organic material degradation and nitrification process. The effects of Zn (II) on nitrification performance were determined by monitoring the bioactivities of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), and effluent Zn (II) concentrations. It was observed that the organic matter removals of SBRs fed with wastewater containing different zinc concentrations show high similarity. Ammonium removal efficiencies (ARE) of reactors operated with a concentration of 2.1, 1.2, and 0.6 mg Zn (II)/L were observed as 88.9±1,7%, 89.8±1.0 % and 81.4±7.3 %, respectively. However, a remarkable effect on nitrate conversion rate (NaCR) was determined. NaCR was 98.3±8.2%, 47.5±11.4%, 27.6±6.7% for a concentration of 2.1, 1.2, and 0.6 mg Zn (II)/L, respectively. Additionally, it was concluded that NOB bioactivity and nitrate conversion rates were improved with increasing Zn (II) concentration. The effluent Zn (II) concentrations were measured as 0.67 mg/L, 0.33 mg/L and 0.07 mg/L for a influent concentration of 2.1, 1.2, and 0.6 mg Zn (II)/L, respectively. Results of the influent and effluent zinc concentrations showed that zinc was adsorbed on bioflocs.

Keywords: Zinc, Activated sludge, Nitrification, Toxicity.

Öz

Bu çalışmada, farklı konsantrasyonlarda Zn (II) içeren üç gerçek endüstriyel atıksu, Zn (II)'nin organik madde bozunması ve nitrifikasyon prosesi üzerindeki etkilerini belirlemek için laboratuvar ölçekli ardışık kesikli biyoreaktör (AKR) ile arıtılmıştır. Zn (II)'nin nitrifikasyon performansı üzerindeki etkisi, amonyum oksitleyen bakterilerin (AOB) ve nitrit oksitleyen bakterilerin (NOB) biyoaktiviteleri ve çıkış suyu Zn (II) konsantrasyonu izlenerek belirlenmiştir. Farklı çinko konsantrasyonları içeren atıksu ile beslenen AKR'nin organik madde giderimlerinin yüksek benzerlik gösterdiği görülmüştür. 2.1, 1.2 ve 0.6 mg Zn (II)/L konsantrasyonunda çalıştırılan reaktörlerin amonyum giderme verimleri (AGV) sırasıyla %88.9±1.7, %89.8±1.0 ve %81.4±7.3 olarak gözlenmiştir. Bununla birlikte, nitrat dönüşüm oranı (NaDO) üzerinde dikkate değer bir etki tespit edilmiştir. NaDO sırasıyla 2.1, 1.2 ve 0.6 mg Zn (II)/L'lik bir konsantrasyon için %98.3±8.2, %47.5±11.4, %27.6±6.7 olmuştur. İlave olarak, Zn (II) konsantrasyonunun artmasıyla NOB biyoaktivitesinin iyileştiği sonucuna varılmıştır. Çıkış suyu Zn (II) konsantrasyonları, sırasıyla 2.1, 1.2 ve 0.6 mg Zn (II)/L'lik bir giriş konsantrasyonu için 0.67 mg/L, 0.33 mg/L ve 0.07 mg/L olarak ölçülmüştür. Giriş ve çıkıştaki çinko konsantrasyonlarının sonuçları, çinkonun biyofloklar üzerinde adsorbe edildiğini göstermiştir.

Anahtar kelimeler: Çinko, Aktif çamur, Nitrifikasyon, Toksisite.

1 Introduction

Due to increasing industrialization and urbanization, the discharge of wastewater containing toxic heavy metals such as lead, chromium, cadmium, zinc, and copper into the receiving environment causes adverse environmental effects on ecosystems and human health [1],[2]. Discharging of the heavy metals in high concentration to the aquatic environment without any treatment is one of the most significant environmental problems for the living organism in receiving media [3]. High metal concentration in wastewater can be also toxic for microorganisms during biological treatment processes [3],[4]. For these reasons, the entrance of heavy metals into the aquatic environment and their effects on biological processes is an issue of great concern in environmental science.

The effects of heavy metals on biological processes vary according to their concentration and type. Certain heavy metals provide an increase in biomass yield at low concentrations, while the same metals cause a decrease in biokinetic parameter values at high concentrations (>5mg/L), by inhibiting microbial growth [5]. For instance, it was observed that Zn (II) concentration inhibited microorganisms in activated sludge above 1 mg/L, but a slight stimulant effect was observed at concentrations below 1 mg/L [5],[6]. Some heavy metals negatively affect biomass growth even at low concentrations. It was assigned to the characterization of activated sludge due to

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the its mixed culture nature. However, various concentrations for inhibiting or inducing of biomass were reported in previous studies due to the using different seed and different operating conditions such as hydraulic retention time (HRT) and pH.

A sequencing batch reactor (SBR) was operated with wastewater containing different Zn (II) concentrations (0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 g/L) to investigate the impact of Zn (II) exposure on the biodegradation ability of activated sludge. It was reported that organic matter removal was not affected up to 400 mg/L Zn (II) concentration [7].

The nitrification process carried out by autotrophic bacteria is the oxidation of ammonium to nitrate and plays a significant role for the removal of nitrogen in biological wastewater treatment plants [8],[9]. Heavy metals such as Ni (II), Zn (II), Cu (II), Cd (II), and Pb (II) entering wastewater treatment plants with industrial discharges can also affect the performance of autotrophic bacteria. Kapoor et al. conducted a study depending on the sOUR (oxygen uptake rate) method to examine the inhibition of nitrification by heavy metals [10]. According to this study, as the dose of Ni (II) and Cd (II) applied to the nitrification biomass increased, the specific ammonium oxidation rate decreased because of exposure. They reported that Zn (II) inhibition on nitrification process rised up to 3 mg/L Zn (II), but it stopped when it exceeds 10 mg/L. In another study, the impact of Cu (II) and Zn (II) on nitrification process by using respirometric analysis, which is an effective technique to define the maximum specific growth rate of autotrophic biomass under toxic conditions, was investigated. It was determined that 0.08 mg/L Cu (II) concentration has a toxic impact on the nitrification by 50%, while Zn (II) inhibited by 12% at the same concentration. The IC50 value for Zn (II) was determined as 0.35 mg/l [11].

Contrary to the studies showing that nitrification inhibition increases with increasing metal concentration, there are also studies showing that it positively affects autotrophic biomass when it is certain concentration ranges in the wastewater. In a study investigating the short-term and long-term effect of Zn (II) on nitrification, it was concluded that low Zn (II) concentration not only had a remedial effect on ammonium oxidizing bacteria (AOB), but also induced nitrite oxidizing bacteria (NOB) bioactivity. While the suppression threshold concentration of Zn (II) was 0.01 g/L for the short-term effect, it reached 0.05 g/L for the long-term effect. This high concentration of Zn (II) for the long-term effect was attributed to self-adaptation of microorganisms [12]. These results demonstrate that the adsorption ability of zinc to the partial nitrification sludge is significant. When a similar study was carried out under anaerobic conditions, it is concluded that the bioactivity of anaerobic ammonium oxidizing bacteria increased in the short term, but nitrogen removal was adversely affected in the long term [13].

In another study, the adsorption capacity of copper, chromium, lead and zinc was investigated to monitor the effects of metal containing wastewater on microbial culture. It has been observed that the adsorption capacity of these metals is much faster than that of organic materials, and they also affect the COD adsorption capacity. According to the results, metal ions inhibited organic adsorption by competing with organic compounds for the active site on the bioflocs instead of showing a toxic effect by inhibiting microbial activity [3],[14]. However, it was observed that the adsorption capacity of zinc to the activated sludge decreased as the initial concentration increased. In another study to investigate the adsorption to dry biomass, the removal efficiency of Zn (II) reduced from 89.0% to 17.6% when the feed concentration was increased from 0.02 to 0.2 g/L [14].

Zn (II) is one of the most common heavy metals in nitrogen-rich industrial wastewater such as leachate, medical wastewater and metal refinery wastewater [2],[14],[15]. Many studies have been published on the impacts of Zn (II) in different wastewater treatment plants, including nitrification-denitrification process [15],[16], methane production [17], Anammox [18], and partial nitrification process [12],[19]. The results of these studies had valuable conclusions about effects of ZN (II) on nitrogen removal techniques. However, limited studies had been focused on effects of different concentration of Zn (II) on the organic material degradation and nitrification process both in the long-term treatment of real industrial wastewater.

In this study, three real industrial wastewaters containing different concentration of Zn (II) were treated with lab-scale SBR. The impacts of different concentration of Zn (II) on the organic material degradation and nitrification process in an activated sludge process was examined. The impacts of Zn (II) on nitrification performance were determined by monitoring the bioactivities of AOB and NOB, and effluent Zn (II) concentrations.

2 Materials and methods

2.1 Wastewater

Three different real industrial wastewaters with Zn (II) concentration of 0.6, 1.2 and 2.1 mg/L used in the study was supplied from an organized industrial zone in Turkey. The wastewater samples were taken from the influent stream of OIZ central wastewater treatment plant at three different times. The main components of these wastewaters were given in Table 1.

Table 1. Characteristics of real industrial wastewaters.

Wastewater	Zn (mg/L)	sCOD (mg/L)	TKN (mg/L)
Wastewater 1	2.1	330	81.2
Wastewater 2	1.2	388	89.6
Wastewater 3	0.6	330	73.7

2.2 Sequencing batch reactor

The experimental set-up of three laboratory-scale SBRs is given in Figure 1, which were operated with real industrial wastewaters. Activated sludge seed was taken from the aeration tank of the organized industrial wastewater treatment plant. Each reactor has a volume of 1 L, and one cycles per day. All SBRs were operated with a sludge retention time (SRT) of 10 days and HRT of 24 hours. The pH of the industrial wastewater was in the range of 8.2 between 8.7 and the pH adjustment was not performed. The F/M ratios were 0.39, 0.38 and 0.38 gSCOD/gVSS for SBR1, SBR2 and SBR3, respectively.

In each cycle process, the reaction phase was determined as 1370 minutes under aerobic conditions with continuous mixing. Feeding was done within the first 5 minutes of the reaction phase. When the reaction phase was over, aeration and mixing were stopped and the settling phase was begun that was planned as 60 minutes. In the last 10 minutes of the cycle, the supernatant was discharged.

In each cycle, while the raw wastewater with a volume of 0.5 L was fed in the SBR, the same amount of effluent was discharged to provide a final volume (1 L) before starting a new cycle. All SBRs were monitored by measuring the concentration of soluble chemical oxygen demand (sCOD), nitrite (NO_2^-), nitrate (NO_3^-), and ammonium (NH_4). The experimental setup of SBRs is shown in Figure 1.

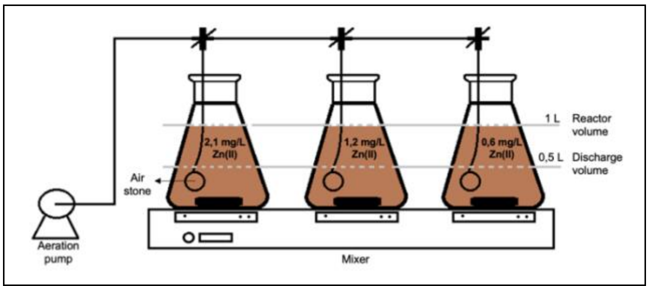

Figure 1. Experimantal set up of lab-scale SBRs.

2.3 Analytical procedures

Conventional parameters (pH, TSS and SCOD) were performed according to Standard Methods [20]. SCOD was analyzed by the closed reflux with the colorimetric method after the samples were filtered. Ammonium concentration was analyzed according to the Standard Methods through the titrimetric method [20]. Nitrite and nitrate measurements were tested by the spectrophotometric method. The pH and conductivity were measured with a multiparameter (Hach-HQ40d). Zn analyses were performed by Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES, Optima 7000 DV, Perkin Elmer, USA).

The SCOD (SCODRE) and ammonium removal efficiency (ARE), nitrite (NiCR) and nitrate conversion ratio (NaCR), nitrite accumulation rate (NAR) were calculated with the equations (1), (2), (3), (4) and (5) respectively.

$$SCODRE = \frac{[SCOD]_{inf.} - [SCOD]_{eff.}}{[SCOD]_{inf.}} \times 100 \tag{1}$$

$$ARE = \frac{[NH_4{}^+ - N]_{inf.} - [NH_4{}^+ - N]_{eff.}}{[NH_4{}^+ - N]_{inf.}} \times 100 \tag{2}$$

$$NiCR = \frac{[NO_2{}^- - N]_{eff.}}{[NH_4{}^+ - N]_{inf.}} \times 100 \tag{3}$$

$$NaCR = \frac{[NO_3{}^- - N]_{eff.}}{[NH_4{}^+ - N]_{inf.}} \times 100 \tag{4}$$

$$NAR = \frac{[NO_2{}^- - N]_{eff.}}{[NO_2{}^- - N]_{eff.} + [NO_3{}^- - N]_{eff.}} \times 100 \tag{5}$$

3 Results

3.1 Zn(II) effect on organic matter removal

The SCOD removal efficiencies of the reactors at the end of 20th day, were determined as 77±3%, 79±5% and 74±9%, respectively. After the completed acclimation period, the efficiencies were found to be 86±2.8%, 84±1.7% and 83±3.1% on average for SBR1, SBR2 and SBR3, respectively. The SCOD removal efficiencies of SBRs are also demonstrated in Figure 2.

The adeptness of the activated sludge to tolerate the toxic impacts of Zn (II) following acclimation with wastewater containing Zn is responsible for the increase in organic matter removal efficiency (II). However, the organic matter removals of SBRs fed with wastewater containing different zinc concentrations show great similarity. These results are consistent with the literature that it is reported the organic removal capacity of activated sludge can withstand up to 400 mg/L zinc concentration without a significant decrease [7]. Tolerance of the activated sludge to the toxic impacts of Zn (II) make possible microorganisms to be used as a biosorbent for Zn (II) removal in biological wastewater treatment processes.

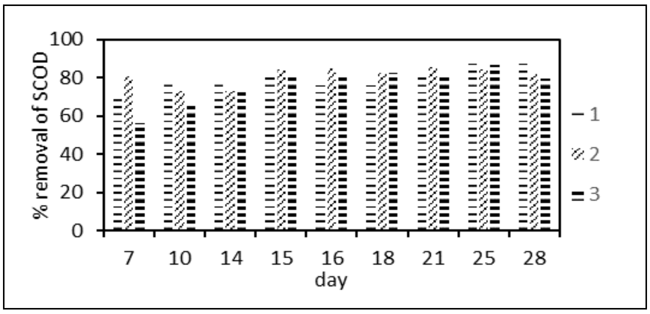

Figure 2. The SCOD removal efficiencies of SBRs.

3.2 Zn(II) effect on nitrification process

Lab-scale SBRs were fed with real industrial wastewater including different concentrations of Zn(II). The reactor performances were determined by monitoring effluent ammonium, nitrite and nitrate measurements to examine the impact of Zn (II) concentration on nitrification. Ammonium, nitrite and nitrate concentrations in the effluent are given for 3 different wastewaters in Figure 3-5.

Ammonium removal efficiencies (ARE) of reactors operated with a concentration of 2.1 mg Zn (II)/L, 1.2 mg Zn (II)/L and 0.6 mg Zn (II)/L were observed as 88.9±1,7%, 89.8±1.0% and 81.4±7.3 %, respectively. Although an increment in Zn (II) (from 0.6 mg/L to 1.2 mg/L) improved the microbial activity of AOB and ammonium oxidation rate, it was observed that Zn (II) content did not have an important effect on ammonium removal efficiencies. However, a remarkable effect on nitrate conversion rate (NaCR) was determined. NaCR was 98.3±8.2%, 47.5±11.4%, 27.6±6.7% for SBR 1, SBR 2 and SBR 3, respectively. Additionally, it was observed that NOB bioactivity was stimulated with increasing Zn (II) concentration, and nitrate conversion rates increased. This result was consistent with the previously report that long-term exposure of Zn (II) stimulates NOB activity [11]. Zhang et al. reported that low Zn (II) (<5 mg/L) were beneficial for AOB activity, though the long-term effect also improved NOB activity [21]. As previous studies stated, Zn (II) was the part of some specific enzymes, so the limited concentration would improve microorganism activity [12],[19].

Contrary to two separate studies where zinc was reported to cause nitrification inhibition at 1.2 mg/L and 3 mg/L concentrations, in this study the zinc concentration was increased up to 2.1 mg/L and it was determined to induce NOB bioactivity [10],[11]. Also results of NaCR were consistent with the nitrite accumulation rates (NAR) in the system. (7.4% for 0.6 mg/L Zn (II), 3.6% for 1.2 mg/L Zn (II), and 0.02% for 2.1 mg/L Zn (II). Thus, it can be established that NOB is more sensitive than AOB to Zn (II) content.

3.3 Adsorption of Zn(II) on biomass

Previous studies reported that low concentrations of zinc compete with organic substances and adsorb to activated sludge at high rates [3].

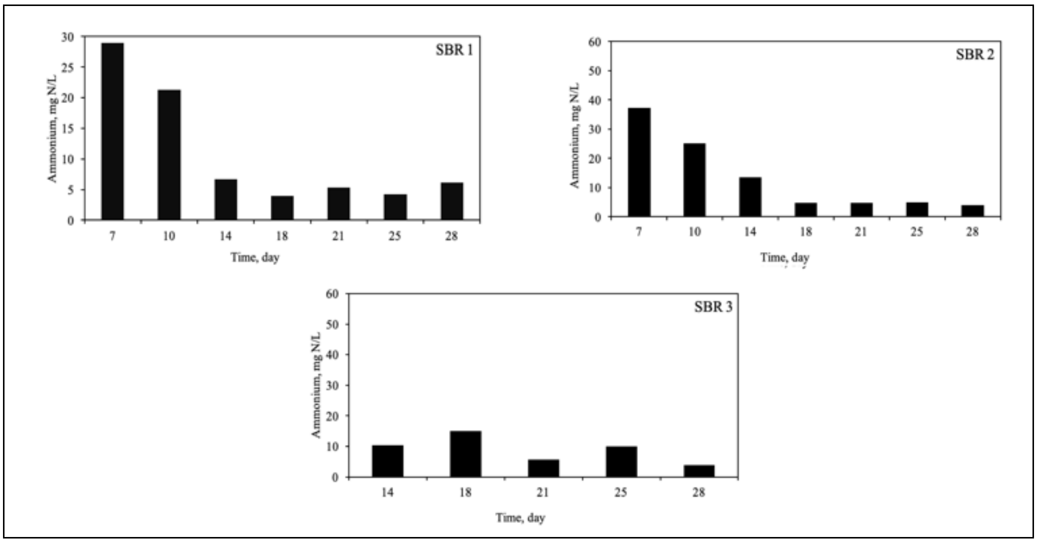

Figure 3. Effluent ammonium concentrations for SBRs.

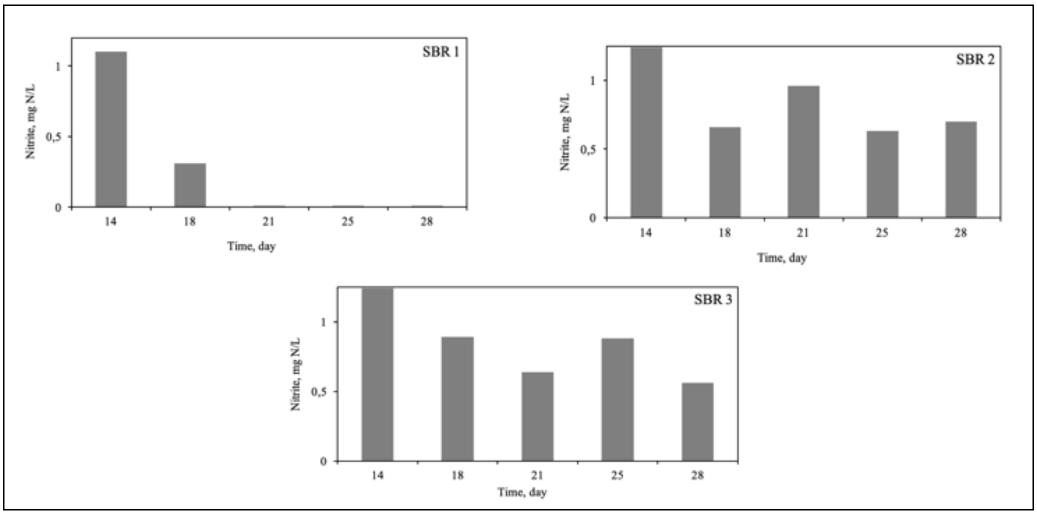

Figure 4. Effluent nitrite concentrations for SBRs.

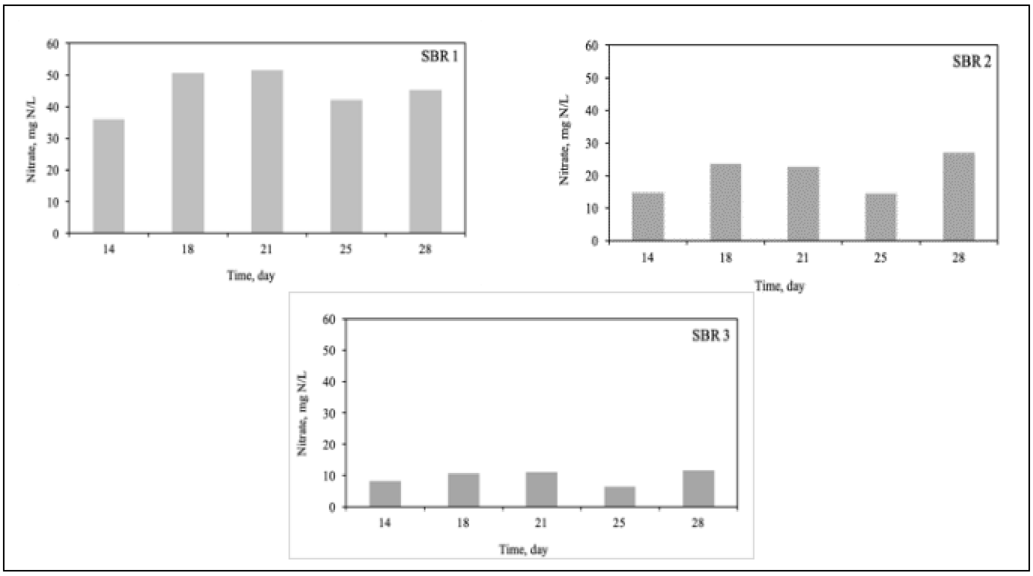

Figure 5. Effluent nitrate concentrations for SBRs.

For this reason, zinc analyzes were carried out in the effluent to determine the fate of zinc in the inlet wastewater. The effluent Zn (II) concentrations of lab scale SBRs were measured as 0.67 mg/L, 0.33 mg/L and 0.07 mg/L for SBR 1, SBR 2 and SBR 3, respectively. Results of the influent and effluent zinc concentrations showed that zinc was adsorbed on bioflocs and thus was largely removed. In addition, these results are compatible with the literature [14]. The adeptness of activated sludge to adsorb zinc makes it possible for microorganisms to be used as a biosorbent for the removal of Zn (II) in biological wastewater treatment processes and can reduce chemicals, energy demand and operating costs.

4 Conclusions

Three industrial wastewaters containing various concentrations of Zn (II) were treated with lab-scale SBR to decide the impacts of various concentrations of Zn (II) on the organic material degradation and nitrification process. The organic content removal efficiencies were observed as 86±2.8%, 84±1.7% and 83±3.1% for SBR1, SBR2 and SBR3, respectively. The improvement of organic matter removal efficiency is attributed to the adeptness of activated sludge to the toxic impacts of Zn (II) by acclimation with wastewater containing Zn (II). ARE of reactors operated with a concentration of 2.1 mg Zn (II)/L, 1.2 mg Zn (II)/L and 0.6 mg Zn (II)/L were determined as 88.9±1,7%, 89.8±1.0% and 81.4±7.3 %, respectively. Although an increment in Zn (II) (from 0.6 mg/L to 1.2 mg/L) improved the microbial activity of AOB and ammonium oxidation rate, it was observed that Zn (II) content did not have an important impact on ammonium removal efficiencies. However, a remarkable effect on nitrate conversion rate (NaCR) was determined. NaCR was 98.3±8.2%, 47.5±11.4%, 27.6±6.7% for SBR 1, SBR 2 and SBR 3, respectively. Additionally, it was observed that NOB bioactivity was stimulated with increasing Zn (II) concentration, and nitrate conversion rates increased. Additionally, the effluent Zn (II) concentrations of SBRs were investigated to decide the adsorption of Zn (II) on biomass. Results of the influent and effluent zinc concentrations shows that zinc was adsorbed on bioflocs and thus was largely removed. Consequently, it was concluded that low Zn (II) concentration enhanced the autotrophic biomass activity and improved nitrate conversion rates.

5 Author contribution statements

Bengisu Cifcioglu-Gozuacik: Investigation, Conceptualization, Writing, Data Curation–original draft. Dilşad Soylu: Investigation. Writing. Bahar Ozbey-Unal: Review & Editing. Cigdem Balcik: Review & Editing. Bulent Keskinler: Conceptualization, Methodology, Supervision.

6 Ethics committee approval and conflict of interest statement

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

7 References

[1] Yang C, Wang J, Lei M, Xie G, Zeng G, Luo S. "Biosorption of zinc (II) from aqueous solution by dried activated sludge". *Journal of Environmental Sciences*, 2(5), 675-680, 2010.

[2] Axtell NR, Sternberg SP, Claussen K. "Lead and nickel removal using Microspora and Lemna minör". *Bioresource Technology*, 89(1), 41-48, 2003.

[3] Chua H, Yu PHF, Sin SN. Cheung, MWL. "Sub-lethal effects of heavy metals on activated sludge microorganisms". *Chemosphere*, 39(15), 2681-2692, 1999.

[4] Codina JC, Perez-Garcia A, De Vicente A. "Detection of heavy metal toxicity and genotoxicity in wastewaters by microbial assay". *Water Science & Technology*, 30(10), 145-151, 1994.

[5] Cabrero A, Fernandez S, Mirada F, Garcia J. "Effects of copper and zinc on the activated sludge bacteria growth kinetics". *Water Research*, 32(5), 1355-1362, 1998.

[6] Dilek FB, Gokcay CF, Yetis U. "Combined effects of Ni (II) and Cr (VI) on activated sludge". *Water Research*, 32(2), 303-312, 1998.

[7] Han JC, Liu Y, Liu X, Zhang Y, Yan YW, Dai RH, Zha XS, Wang CS. "The effect of continuous Zn (II) exposure on the organic degradation capability and soluble microbial products (SMP) of activated sludge". *Journal of Hazardous Materials*, 244, 489-494, 2013.

[8] Shi XY, Sheng GP, Li XY, Yu HQ. "Operation of a sequencing batch reactor for cultivating autotrophic nitrifying granules". *Bioresource Technology*, 101(9), 2960-2964, 2010.

[9] Duyar A, Ciftcioglu V, Civelekoglu G, Cirik K. "Performances of sequential denitrification and partial nitrification process for treatment of landfill leachate". *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi*, 27(6), 737-743, 2021.

[10] Kapoor V, Li X, Elk M, Chandran K, Impellitteri CA, Santo Domingo JW. "Impact of heavy metals on transcriptional and physiological activity of nitrifying bacteria". *Environmental science & technology*, 49(22), 13454-13462, 2015.

[11] Juliastuti SR, Baeyens J, Creemers C, Bixio D, Lodewyckx, E. "The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge". *Journal of hazardous materials*, 100(1-3), 271-283, 2003.

[12] Zhang X, Zhou Y, Zhang N, Zheng K, Wang L, Han G, Zhang H. "Short-term and long-term effects of Zn (II) on the microbial activity and sludge property of partial nitrification process". *Bioresource Technology*, 228, 315-321, 2017.

[13] Zhang X, Chen Z, Zhou Y, Ma Y, Ma C, Li Y, Liang Y, Jia J. "Impacts of the heavy metals Cu (II), Zn (II) and Fe (II) on an Anammox system treating synthetic wastewater in low ammonia nitrogen and low temperature: Fe (II) makes a difference". *Science of the Total Environment*, 648, 798-804, 2019.

[14] Yang C, Wang J, Lei M, Xie G, Zeng G, Luo S. "Biosorption of zinc (II) from aqueous solution by dried activated sludge". *Journal of Environmental Sciences*, 22(5), 675-680, 2010.

[15] Çeçen F, Semerci N, Geyik AG. "Inhibitory effects of Cu, Zn, Ni and Co on nitrification and relevance of speciation". *Journal of Chemical Technology & Biotechnology*, 85(4), 520-528, 2010.

[16] Feng B, Fang Z, Hou J, Ma X, Huang Y, Huang L. "Effects of heavy metal wastewater on the anoxic/aerobic-membrane bioreactor bioprocess and membrane fouling". *Bioresource Technology*, 142, 32-38, 2013.

[17] Madoni P, Davoli D, Guglielmi L. "Response of SOUR and AUR to heavy metal contamination in activated sludge". *Water Research*, 33(10), 2459-2464, 1999.

[18] Zhang ZZ, Zhang QQ, Xu JJ, Deng R, Ji ZQ, Wu YH, Jin RC. "Evaluation of the inhibitory effects of heavy metals on anammox activity: a batch test study". *Bioresource Technology*, 200, 208-216, 2016.

[19] Daverey A, Chen YC, Sung S, Lin JG. "Effect of zinc on anammox activity and performance of simultaneous partial nitrification, anammox and denitrification (SNAD) process". *Bioresource Technology*, 165, 105-110, 2014.

[20] Baird R, Bridgewater L. *American Public Health Association. Standard Methods for the Eamination of Water and Wastewater*. 23th ed. Washington, DC, American public Health Association 2017.

[21] Zhang X, Chen Z, Zhou Y, Ma Y, Ma C, Li Y, Liang Y, Jia J. "Impacts of the heavy metals Cu (II), Zn (II) and Fe (II) on an Anammox system treating synthetic wastewater in low ammonium nitrogen and low temperature: Fe (II) makes a difference". *Science of the Total Environment*, 648, 798-804, 2019.