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Long-term effect of copper and tetracycline on the performance and sludge characteristics of anoxic-aerobic sequencing batch reactor with integrated sludge disintegration

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ABSTRACT

In this study, it was aimed to determine the change in the toxic effect of copper (Cu^{2+}) and tetracycline (TC) on biological treatment by sludge disintegration application. For this purpose, two lab-scale reactors, sequencing batch reactor (SBR) and sludge disintegration applied sequencing batch reactor (SBR-SD), were used. In the control stage of SBR and SBR-SD, namely SBR-C and SBR-SD-C, the chemical oxygen demand (COD) and NH_4^+–N removal efficiency was 93% and 95%, respectively. While reductions were observed in the COD and NH_4^+–N removal efficiency from the 66th day in SBR, stable treatment efficiency was obtained in SBR-SD throughout the operation. While sludge volume index value increased to 175 mL/g in SBR, it remained stable between 78–85 mL/g in SBR-SD. While the decrease in the specific oxygen consumption rate value in SBR was 55% on the 90th day due to the toxicity of Cu^{2+} and TC, it was 29% in SBR-SD. In SBR, PN and PS content in tightly bound extracellular polymeric substances (TB-EPS) and loosely bound extracellular polymeric substances (LB-EPS) increased due to resistance to the toxic effects of Cu^{2+} and TC. The protein (PN) and polysaccharide (PS) content in TB-EPS and LB-EPS remained the same as the control stage values since the toxic effect of Cu^{2+} and TC in SBR-SD was relatively eliminated by sludge disintegration. The toxic effect of Cu^{2+} and TC on biological treatment was prevented by sludge disintegration and stability was ensured during long-term operation.

Keywords: Copper; Tetracycline; Sequencing batch reactor; Sludge disintegration

1. Introduction

Copper is a heavy metal that is required in trace amounts for animal growth [1]. Tetracycline is an antibiotic used for the prevention and treatment of diseases in animals [2]. Therefore, copper and tetracycline are found together in livestock and poultry wastewater [3]. In addition, high concentrations of copper and tetracycline are found in wastewater generated in the manufacturing industry and pharmaceutical industry, respectively. In cases where the industrial wastewater is not treated separately, these wastewaters can be given to the treatment plants where the municipal wastewater is treated. The effective mechanism of copper removal from wastewater by biological methods is adsorption. In tetracycline removal, the most basic mechanism is adsorption, although the effective mechanisms are biodegradation and adsorption. Adsorption is the transition of pollutants from one phase (liquid) to another phase (solid). Copper and tetracycline can be adsorbed better by forming a complex together, and its effect on microorganisms differs from the single effect of copper and tetracycline [4]. Activated sludge has a certain adsorptive capacity and copper and tetracycline can be released from the activated sludge in long-term plant operation and discharged to the receiving environment with the effluent of the treatment plant. In addition, the effects of copper

and tetracycline accumulated in the sludge on the sludge treatment processes and the threats that may occur if they are discharged into the receiving environment are also ignored. *In-situ* treatment of sludge has become popular in recent years. Among the strategies developed for this, the lysis-cryptic growth method is preferred primarily because it is more applicable. The sludge lysis process is carried out by chemical, mechanical, thermal and biological methods [5]. Fenton method is one of the methods used for sludge lysis. However, the fact that this method is more effective at low pH and then requires pH adjustment limits the applicability of the method. In recent years, different metals have been tried instead of iron(II) as a catalyst in the Fenton method. Among these metals, many studies have been conducted on copper because it is more effective at neutral pH. H_2O_2 is an environmentally friendly oxidant that does not form toxic by-products. However, H_2O_2 has a low redox potential and when used alone, sufficient oxidation does not occur. H_2O_2 is also used in sludge disintegration [6]. In this study, it was aimed to use the copper released from the sludge as a catalyst by using H_2O_2 for sludge disintegration and to remove the tetracycline released from the sludge as a result of its reaction with H_2O_2 in the environment. Thus, the change in the toxic effect of copper (Cu^{2+}) and tetracycline (TC) on biological treatment will also be determined.

2. Materials and methods

2.1. Reactor operation and wastewater

Two lab-scale sequencing batch reactors were used in this study. In the sequencing batch reactor (SBR) and sludge disintegration applied sequencing batch reactor (SBR-SD), copper and tetracycline were not added for 18 d. After 18 d in SBR, the addition of copper and tetracycline to the reactor and in SBR-SD, both tetracycline and copper addition and sludge disintegration were started. Synthetic wastewater was supplied to the reactors with the help of a peristatic pump. In the anoxic phase, the wastewater was mixed with a mechanical mixer. In the aerobic phase, aeration was carried out by an air pump and a diffuser placed at the bottom of the reactor. While the dissolved oxygen concentration was below 0.5 mg/L in the anoxic phase, the dissolved oxygen concentration was between 2–3 mg/L in the aerobic phase. In SBR-SD, 4% of the reactor content was taken at the end of the discharge phase and subjected to sludge disintegration. Sludge disintegration was carried out at pH 8 and 100 mg/L concentration of H_2O_2 as a result of the results of preliminary laboratory studies. The sludge was reacted for 1 h and the disintegrated sludge was recycled back to the sequencing batch reactor.

The components contained in each liter of synthetic wastewater fed to the reactors are: $CH_3COONa{\cdot}3H_2O$ (641 mg), NH_4Cl (114.6 mg), KH_2PO_4 (8.5 mg), K_2HPO_4 (9.4 mg), $NaHCO_3$ (112.5 mg), $ZnSO_4{\cdot}7H_2O$ (0.12 mg), H_3BO_3 (0.15 mg), $FeCl_3{\cdot}6H_2O$ (01.5 mg), $MnCl_2{\cdot}4H_2O$ (0.12 mg), KI (0.03 mg), $NiSO_4$ (0.15 mg), $CoCl_2{\cdot}6H_2O$ (0.15 mg), $Na_2MoO_4{\cdot}2H_2O$ (0.06 mg). The influent of sequencing batch reactors contains 20 mg/L Cu^{2+} and 10 mg/L TC considering the literature data.

2.2. Analytical methods

Chemical oxygen demand (COD), total suspended solids (TSS), sludge volume index (SVI) and specific oxygen consumption rate (SOUR) analyzes were performed according to Standard Methods [7]. Ammonium (Kit Code: 100683), nitrite (Kit Code: 114776) and nitrate (Kit Code: 114773) analyzes were performed using the Standard Kit (Merck Specquorant, Nova 60, Germany). The loosely bound extracellular polymeric substances (LB-EPS) and tightly bound extracellular polymeric substances (TB-EPS) contents of the sludge were determined by modified heat extraction method [8]. Carbohydrate and protein content of each extracellular polymeric substrate was determined by phenol sulfuric acid and Lowry method, respectively [9,10].

2.3. Calculation of sludge product and sludge reduction efficiency

Observed sludge product (Y_{obs}) was calculated using Eq. (1) given earlier [11].

$$Y_{obs}\left(\text{g SS/gCOD}\right)=\frac{W}{Q\times\left(\text{KO0}_i-\text{KO0}_e\right)} \tag{1}$$

where W is excess sludge produced in the reactor g·AKM/d; Q, flow rate m^3/d; COD_i and COD_e are the concentrations of COD entering and leaving the reactor, respectively, mg/L.

Sludge reduction efficiency (SRE) was found using Eq. (2) [11].

$$\text{SRE}=\left(1-\frac{Y_{obs}-d}{Y_{obs}-c}\right)\times 100 \tag{2}$$

where $Y_{obs\text{-}d}$, sludge product observed by sludge disintegration, g·SS/g·COD; $Y_{obs\text{-}c}$ shows the observed sludge product without sludge disintegration, g·SS/g·COD.

3. Results and discussion

3.1. Comparison of treatment performances

The COD and NH_4^+–N removal efficiency of SBR and SBR-SD in the control stage without Cu^{2+} and TC added in the 0–18 d time interval is 93% and 95%, respectively (Fig. 1). With the addition of Cu^{2+} and TC in the 18–24 d time period, the COD and NH_4^+–N removal efficiency in both reactors decreased to 86% and 85%, respectively. These results show that Cu^{2+} and TC have toxic effects on activated sludge [1,12]. After one week, the COD and NH_4^+–N removal efficiency increased to 90% and 88%, respectively, as a result of the adaptation of the activated sludge. In SBR-SD, stability was achieved in terms of COD and NH_4^+–N removal throughout the whole operation. This showed that the toxic effects of Cu^{2+} and TC could be tolerated during long-term operation in SBR-SD where sludge disintegration was integrated. From the 66th day, the COD and NH_4^+–N removal efficiency started to decrease in SBR. These results were related to the inhibition of activated sludge and the decrease in its concentration [4]. At the 0–18 d control stage, the effluent NO_3^-–N

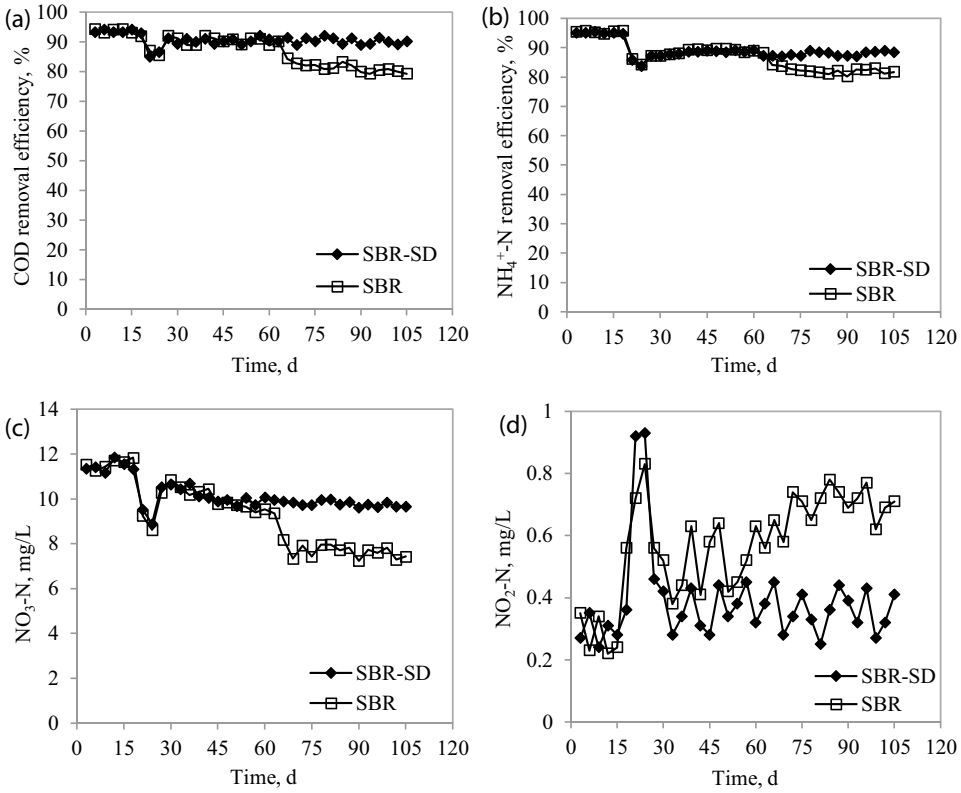

Fig. 1. Effect of Cu^{2+} and TC on SBR and SBR-SD performance: (a) chemical oxygen demand, (b) NH_4^+–N, (c) NO_3^-–N, and (d) NO_2^-–N.

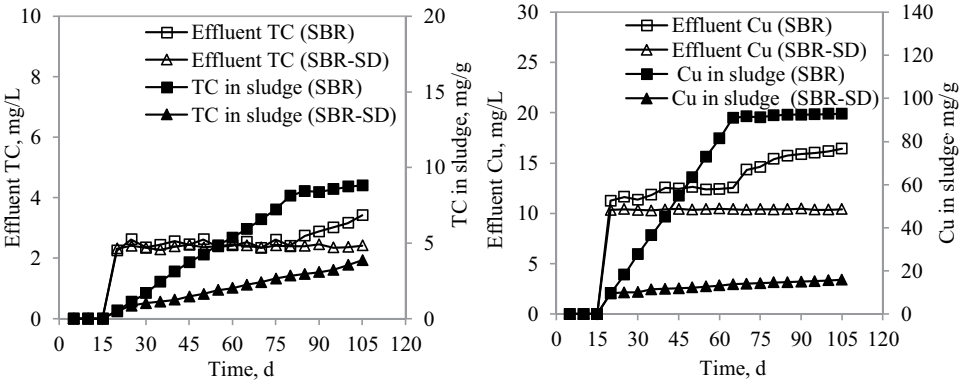

Fig. 2. TC and Cu^{2+} removal in SBR and SBR-SD.

concentration in SBR and SBR-SD was 11.58 and 11.45 mg/L, respectively. With the addition of Cu^{2+} and TC between 18–24 d, the effluent NO_3^-–N concentration decreased to 8.93 and 9.2 mg/L, respectively. As a result of the adaptation of activated sludge to Cu^{2+} and TC toxicity, the effluent NO_3^-–N concentration in SBR and SBR-SD remained stable. However, from day 66 on SBR, the effluent NO_3^-–N concentration decreased to 7.64 mg/L. This decrease indicated that Cu^{2+} and TC inhibited the nitrification process. It was stated that the inhibitory effect of Cu^{2+} and TC was concentration and time dependent [13]. Although the effluent NO_2^-–N concentration was relatively higher in SBR compared to SBR-SD, no accumulation was observed in the NO_2^-–N concentration.

3.2. Role of bioadsorption and biodegradation in copper and tetracycline removal

Between 20–70 d, while the effluent TC concentration in SBR was constant in the range of 2.25–2.62 mg/L, its concentration started to increase with advancing operating times (Fig. 2). The increase in the effluent TC concentration in SBR was related to the inhibition of the activated sludge and the filling of the sludge adsorption capacity. It was stated that excessive accumulation of chemicals on the aerobic granule will cause release in the saturated and balanced system [14]. In SBR-SD, the effluent TC concentration was in the range of 2.32–2.46 mg/L and its concentration remained stable throughout the whole operation. In SBR, the TC

concentration in the sludge increased during the operation period, and then a stable state was formed. It was seen that the increase of TC concentration in the sludge in SBR-SD was less compared to SBR. In SBR-SD, both Cu^{2+} and TC concentrations in sludge decreased due to sludge disintegration. It is seen that Cu^{2+} released from the sludge acts as a catalyst by reacting with H_2O_2 and the TC released from the sludge can be removed with the products formed as a result of this reaction. In the Cu(II)/H_2O_2 process, it was stated that •OH and Cu(III) coexist and break down sludge flocs and cells [15]. This delayed the filling of the adsorptive capacity of the sludge and provided stability in terms of effluent Cu^{2+} and TC concentrations throughout the operation.

3.3. Sludge reduction and characteristics

The sludge product (Y_{obs}) observed in SBR and SBR-SD at day 15 are 0.37 and 0.38 g·SS/g·COD (Fig. 3). Mixed liquor suspended solids (MLSS) concentration in SBR and SBR-SD was 3,450 and 3,400 mg/L, respectively. As a result of inhibition of activated sludge in SBR due to the addition of Cu^{2+} and TC, sludge product and MLSS concentrations decreased. The toxic effect of Cu^{2+} and TC was relatively eliminated with the sludge disintegration application in SBR-SD and a sludge reduction efficiency of 36% was obtained. Therefore, while a decrease was observed in MLSS concentration due to toxicity in SBR, sludge formation was reduced by sludge disintegration in SBR-SD.

The SVI is a parameter characterizing sludge settling. If the SVI value is between 50–150 mL/g, it means that the sludge settling performance is good, and values greater than 150 mL/g indicate that there is sludge swelling [16]. The SVI values in SBR-C and SBR-SD-C were 70 and 74 mL/g, respectively. This indicated that the sludge was well flocculated and settled. With the addition of Cu^{2+} and TC in SBR, the SVI value increased to 175 mL/g depending on the operating time. This result showed that Cu^{2+} and TC disrupted the cell structures and activities of bacteria [16]. SVI value in SBR-SD ranged between 78–85 mL/g. This result was proportional to the removal of TC as a result of the reaction of Cu^{2+} released by sludge disintegration with H_2O_2 and the reduction of the toxicity of Cu^{2+} and TC to bacteria.

In order to determine the effect of Cu^{2+} and TC on microbial activity, SOUR was investigated at 15, 45, 75 and 90 d in SBR and SBR-SD. SOUR values are similar in SBR-C and SBR-SD-C. With the addition of Cu^{2+} and TC, SOUR decreased by 30%, 42% and 55% in SBR at 45, 75 and 90 d, respectively. In a similar study, SOUR decreased by 42.03% with the addition of Cu^{2+} and TC in a SBR [12]. On the other hand, SOUR values in SBR-SD where sludge disintegration was integrated decreased by 10%, 21% and 29% at 45, 75 and 90 d, respectively. It was observed that the toxicity of

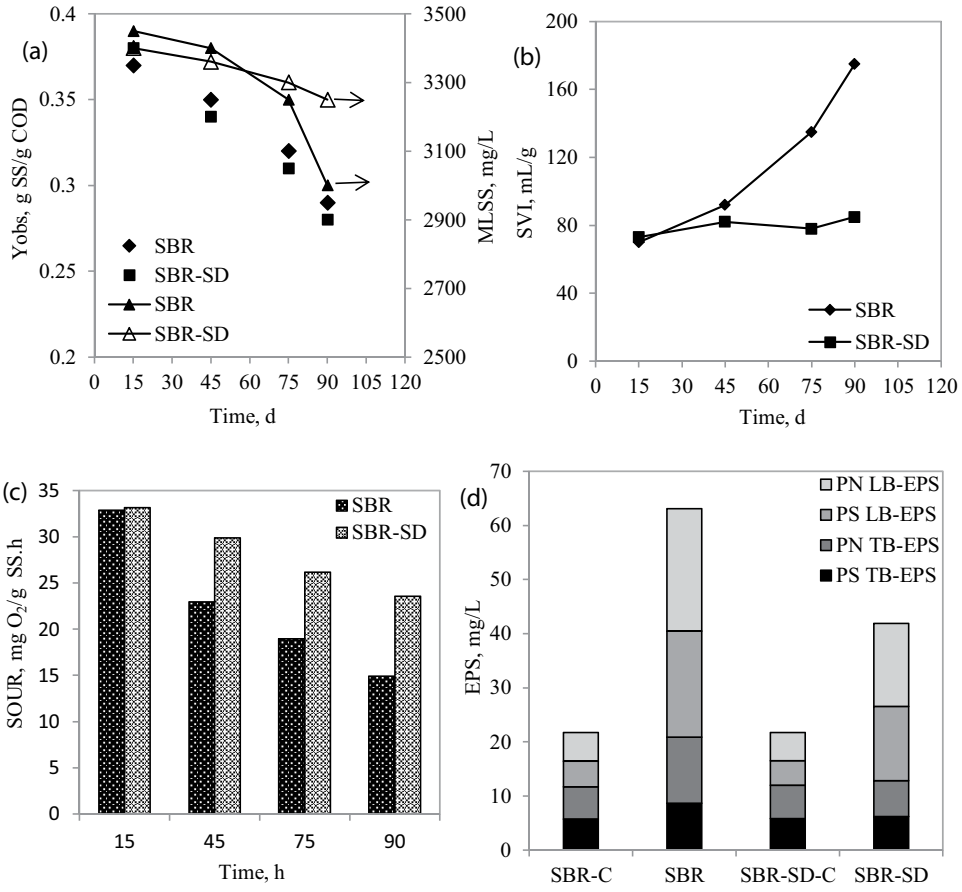

Fig. 3. Sludge reduction and characteristics in SBR and SBR-SD: (a) Y_{obs}, (b) sludge volume index, (c) specific oxygen consumption rate, and (d) extracellular polymeric substances.

Cu^{2+} and TC to activated sludge decreased with the sludge disintegration application.

Extracellular polymeric substances (EPS) are mainly composed of polysaccharide and protein. In SBR-C without Cu^{2+} and TC additions, the protein (PN) and polysaccharide (PS) contents in TB-EPS were 5.95 and 5.74 mg/L, respectively. The PN and PS content in LB-EPS were 5.26 and 4.74 mg/L, respectively. In SBR without sludge disintegration, PN and PS contents in TB-EPS due to the addition of Cu^{2+} and TC increased to 12.24 and 8.68 mg/L, respectively. The PN and PS content in LB-EPS increased to 22.67 and 19.54 mg/L, respectively. The PN and PS contents of EPS increased due to the protective response of bacteria to the increase in Cu^{2+} and TC concentrations. At the same time, due to the increase in Cu^{2+} and TC concentrations, the integrity of the cell membrane was disintegrated and PN and PS were released out of the cell. In SBR-SD-C without Cu^{2+} and TC addition, the PN and PS contents in TB-EPS are 6.12 and 5.82 mg/L, respectively. The PN and PS content in LB-EPS were 5.16 and 4.62 mg/L, respectively. With the addition of Cu^{2+} and TC, the contents of PN and PS in TB-EPS in SBR-SD are 6.58 and 6.24 mg/L, respectively. The PN and PS content in LB-EPS are 15.32 and 13.74 mg/L, respectively. As a result of the interaction of Cu^{2+} and TC released as a result of sludge disintegration in SBR-SD with H_2O_2 in the environment, their concentrations and toxicity in wastewater decreased and the increase in PN and PS contents in EPS remained limited. The PN/PS ratios in SBR-C and SBR-SD-C were similar. The PN/PS ratio in TB-EPS in SBR-C and SBR-SD-C was 1.03 and 1.05, respectively. The PN/PS ratio in LB-EPS in SBR-C and SBR-SD-C was 1.1 and 1.1, respectively. After the control stage in SBR, the PN/PS ratio in TB-EPS increased to 1.4 and the PN/PS ratio in LB-EPS increased to 1.16. Depending on the increase in Cu^{2+} and TC concentrations, the PN/PS ratio increased. The increase in Cu^{2+} and TC concentrations showed that the defense mechanism in EPS was with an increase in protein. This was similar to previous results [1,17]. The PN/PS ratio in SBR-SD remained the same as the values in the control stage. This showed that the toxicity of Cu^{2+} and TC to bacteria was reduced by the application of sludge disintegration.

4. Conclusions

As a result of long-term exposure to Cu^{2+} and TC, there were decreases in COD and NH_4^+–N removal efficiency and MLSS concentration in SBR. In SBR-SD, in which sludge disintegration was integrated, stability was ensured in COD and NH_4^+–N removal efficiency and MLSS concentration even during long-term operation. Activated sludge was inhibited in SBR after prolonged exposure to Cu^{2+} and TC. In SBR-SD, sludge reduction efficiency of 36% was achieved as a result of sludge disintegration. The Cu^{2+} released from the sludge in SBR-SD was evaluated as a catalyst, and its toxicity to the activated sludge was prevented. With the reaction of Cu^{2+} and H_2O_2, TC was also removed by chemical method and its toxic effect on biological treatment was eliminated.

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