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Effect of copper nanoparticles on biological wastewater treatment

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ABSTRACT

The widespread use of nanoparticles (Nps) inevitably leads to their release into the environment, in particular from wastewater treatment plants. During wastewater treatment, nanoparticles undergo aggregation, sedimentation and transformation, which may cause their concentration in effluents and the sludge. Activated sludge contains microorganisms that play an important role in the biological wastewater treatment process. Microorganisms in biological treatment processes may be exposed to nanoparticles that were agglomerated or adsorbed to extracellular polymers during treatment. The aim of the study was to determine the effect of copper nanoparticles (CuNPs) on wastewater treatment in the sequencing batch reactor (SBR) bioreactor. Analyses of total organic carbon (TOC), suspended solids, volatile suspended solids, pH and total copper were performed. Microbial observations of activated sludge were performed and, on their basis, the Madonia sludge biotic index was evaluated. It was found that at a concentration of 3 mg/L CuNPs, the degree of wastewater treatment decreased from 92.17% to 71.9% (based on the TOC). CuNPs may negatively affect the activity of activated sludge in SBR reactors.

Keywords: Nanoparticles; Copper; Wastewater treatment; Sewage sludge

1. Introduction

One of the fastest-growing fields of science is nanotechnology, which uses structures with dimensions from 1 to 100 nm. The Woodrow Wilson Database contains nanomaterials with copper nanoparticles used in the production of cosmetics, supplements, cleaning filters and electronics [1–3]. Copper effectively inhibits the growth of bacteria, fungi, viruses and algae. Therefore, the antibacterial properties of copper nanoparticles are increasingly utilized [4–7]. The more common use of nanoparticles causes their penetration into the environment, with one of the ways being sewage treatment systems [8]. There is also growing concern that the released nanoparticles adversely affect the sewage treatment processes, due to their settlement, biosorption and other processes involving activated sludge microorganisms [9–11]. Research has been conducted on their potential inhibition and toxic effects on microorganisms in biological sewage treatment processes. Due to their properties, size, surface and environmental factors, the interactions and biological effects of their introduction are not fully known [12–14]. It is possible that metal nanoparticles can enter streams, rivers and water reservoirs with treated wastewater. They also get to sewage sludge and landfill sites from where they are transferred to leachate [15,16]. The wide use of nanoparticles poses new unknown threats to humans and the environment [17].

It was found that copper nanoparticles can change the physicochemical properties of activated sludge. Chen et al. [18] studied the use of CuNPs with the content of 0.1–10 mg/L and found that they did not impact in the long term on the removal of nitrogen from wastewater. The research demonstrated that nearly all copper nanoparticles were absorbed by activated sludge, which was not damaged.

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In other studies, deterioration in the flocculation capacity of activated sludge was observed at a higher concentration of 30–50 mg/L CuNPs. The toxicity of copper nanoparticles to bacteria was found [19].

Studies on the long-term effect of copper nanoparticles on biological phosphorus removal showed significant deterioration in its removal. The long-term exposure to CuNPs at a concentration of 50 mg/L caused both a decrease in bacterial diversity and bacterial count. The studies indicated that bacteria sensitive to copper nanoparticles were washed away, whereas the bacteria that removed phosphorus from the sewage were still present [18].

The aim of the present study was to determine the effect of copper nanoparticles (CuNPs) concentration of 3 mg/L on wastewater treatment in the sequencing batch reactor (SBR) reactor. Changes in the removal of pollutants from wastewater on the basis of the total organic carbon (TOC) value and the effect of nanoparticles on activated sludge were analyzed.

2. Materials and methods

2.1. Characterization of test substrates

Synthetic wastewater was used in the study, prepared according to the modified procedure [20]. The wastewater was prepared with the use of glucose and the following microelements: NH_4Cl – 4.5 g/L, K_2HPO_4 – 4.5 g/L, $MgSO_4$ – 1.95 g/L, NaCl – 9 g/L, 1 $CaCl_2$ – 0.45 g/L and K_2HPO_3 g/L. Each time fresh synthetic wastewater was prepared.

2.2. Copper nanoparticles

Copper nanoparticles (CuNPs) from Sigma-Aldrich, (USA) were used. Nanoparticles were in the form of particles with a size below 50 nm. A suspension of CuNPs was obtained by dissolving 1 g of CuNPs in 1 L of Milli-Q water (Merck KGaA, Germany). It was then sonicated for 1 h using Sonics Vibra-Cell VCX 134, USA (130 W, 40 kHz).

2.3. Conditions for biological treatment of wastewater

The study was carried out in two SBR bioreactors (one as a control). Bioreactors with a working volume of 3.5 L were used and mixing was carried out with the blades (rotational speed 50 rpm). Air to the bioreactors was supplied by diffusers installed in the bottom of the tank. During the aeration phase, dissolved oxygen remained at a level of 5 mg/L. The examinations were conducted at room temperature.

A 12 h work cycle was applied. There were five phases for each cycle. The first phase was filling and mixing, lasting 1.0 h. The second phase was mixing and aeration for 8.5 h, the third – mixing for 1.0 h, the fourth – settling and decanting for 1 h, and the fifth (stopping phase) lasted 0.5 h.

Activated sludge was collected from the municipal sewage treatment plant in Częstochowa, Poland, and acclimatized by adding synthetic sewage. The examinations lasted 40 d. After one month, copper nanoparticles were added, with a concentration of 3.0 mg/L. Bioreactors were operating with 12 h sludge retention time and 4 h hydraulic retention time.

2.4. Analytical methods

The following parameters were analyzed in wastewater: TOC, suspended solids (SS), volatile suspended solids (VSS), pH and total copper. TOC was analyzed using multi N/C 3100 as organic carbon remaining in the acidified sample after gas treatment of the sample. SS, VSS and pH were measured according to APHA [21]. The concentration of copper was evaluated by means of atomic absorption spectrometry using the SpectroAcros ICP-OES spectrometer, (Thermo Apparatus, USA).

Microorganisms of activated sludge were observed in the study to determine the sludge biotic index (SBI) by Madoni [22]. This index describes the sensitivity of different groups of microorganisms to the conditions of sewage treatment using activated sludge. Based on the appropriate key, the activated sludge class is determined, whereas the SBI index ranges from 1 to 10 [22].

2.5. Statistical analysis

The statistical estimation was carried out using STATISTICA software (STATISTICA 13.3, TIBCO Software Inc., Palo Alto, CA, USA). The influence of copper nanoparticles on the efficiency of removal of contaminants expressed as TOC and changes in CuNPs concentration in the outflow from the SBR bioreactor was estimated based on the analysis of ANOVA (homogeneity of variances). In the case of statistically significant data ($p < 0.05$) a post hoc Tukey honest significant difference was used. The statistical analysis was to determine whether the applied treatment method had a statistically significant effect on the examined parameters. The results marked with the same letter in a specific group are not statistically significant (different letters a, b, c, d, e in the figures).

3. Results and discussion

3.1. Effect of copper nanoparticles on sewage treatment in the SBR reactor

The examinations were carried out in two sequential biological reactors, which were marked Cu-SBR (bioreactor to which CuNPs were added) and C-SBR (control bioreactor). For 30 d, the bioreactors operated without nanoparticles to obtain acclimatization of activated sludge at a TOC concentration of 180 mg/L. In the next stage of the study, the Cu-SBR bioreactor was dosed with 3 mg/L copper nanoparticles for 10 d, whereas the C-SBR reactor became a control reactor.

Fig. 1 shows the results of TOC removal in the analyzed bioreactors. As can be seen, during the activated sludge acclimatization stage, the TOC concentration at the outflow from bioreactors was at a similar level. In the Cu-SBR reactor, the initial concentration of approximately 180 mg/L declined to values between 15.84 and 23.27 mg/L, while in the C-SBR reactor, it fell to between 13.72 and 27.84 mg/L. However, with the addition of copper nanoparticles, the TOC concentration at the outflow from Cu-SBR began to rise to 59.25 mg/L from day 31 of operation. Furthermore, this tendency was not observed in the case of the control bioreactor. A clear effect of copper nanoparticles on the sewage treatment process was observed.

Analysis of the level of TOC removal from the treated wastewater reveals that it was at a similar level in both

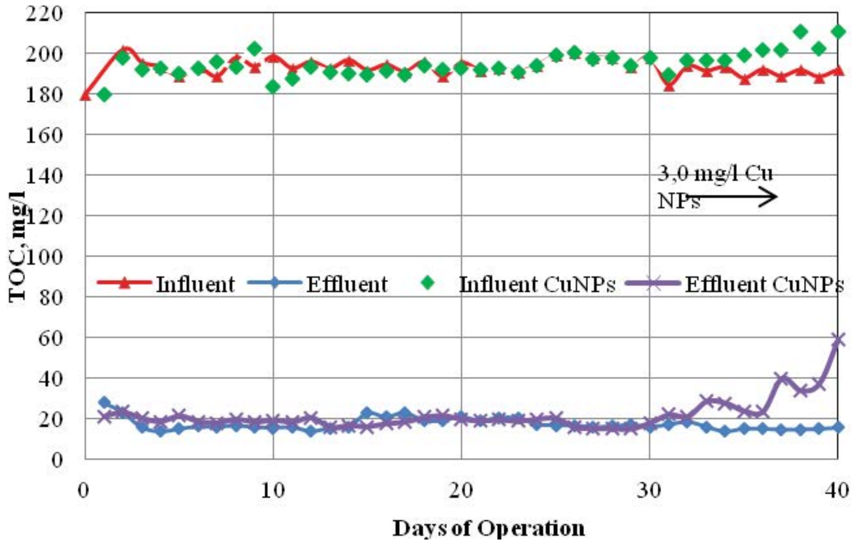

Fig. 1. Changes in TOC concentration during wastewater treatment in Cu-SBR and C-SBR reactors.

bioreactors at the stage of acclimatization, with 88.23%–92.40% for the Cu-SBR bioreactor and from 84.53% to 92.18% for C-SBR (Fig. 2). After the introduction of CuNPs nanoparticles, a clear decrease in the treatment efficiency was observed in the Cu-SBR bioreactor (to 71.9%).

Zhang et al. [23] demonstrated that short-term exposure to CuO NPs at a concentration of 1 mg/L may affect the reduction in chemical oxygen demand (COD) and ammonia. However, the use of higher concentrations of 10 and 50 mg/L caused the inhibition of biological wastewater treatment. Furthermore, Wang et al. [24] studied the effect of CuO NPs nanoparticles with a concentration of 0.10 mg/L and obtained COD removal from wastewater at 91.26%. After a significant increase in the CuO NPs concentration to 30 mg/L, the COD removal rate decreased slightly to 89.48%.

The present study analyzed changes in the content of SS and VSS. Their values are shown in Fig. 3. The SS and VSS contents were observed to stabilize from 10 to day 25 of the activated sludge acclimatization cycle. After adding CuNPs to the dosed wastewater at a concentration of 3 mg/L in the Cu-SBR bioreactor, a slight decrease in SS and VSS was observed in relation to the values in the control bioreactor (C-SBR). It seems that the presence of copper nanoparticles in wastewater reduces the rate of removal of contaminants. At lower values of SS, the organic load on the activated sludge increased, translating into a lower reduction of wastewater contaminants in the SBR reactor.

Fig. 4 shows the changes in pH during the sewage treatment process in the SBR reactors. The pH values during the acclimatization of activated sludge ranged from 6.33 to 6.96. A slight downward trend in pH values was observed during the process. After the addition of copper nanoparticles in the Cu-SBR bioreactor, a downward trend was observed. However, this was not a large decrease and, therefore, at this stage of the study, it would be difficult to conclude that the change in pH value was related to exposure to nanoparticles.

The SBI was evaluated based on the microscopic analysis of activated sludge from the tested bioreactors. In the sludge acclimatization phase, values of 9 were obtained for SBI, which qualified the activated sludge from Cu-SBR and C-SBR bioreactors to the first class according to Madoni et al. [25]. Similar results were obtained by adding silver nanoparticles to the wastewater treated in the SBR reactor [26]. Observations showed that activated sludge was very well colonized and stable, with good biological activity. After the addition of copper nanoparticles to the Cu-SBR bioreactor, changes were observed in the dominant microbial communities responsible for the effectiveness of the activated sludge. SBI decreased to the level of 7 and 2nd class of sludge activity. This could have caused a lower capacity of the activated sludge to remove contaminants.

Fig. 5 presents a list of dominant microorganisms colonizing activated sludge in Cu-SBR and C-SBR bioreactors during their operation in the period after the sludge acclimatization in the bioreactors. Sludge observations showed that testate amoebae accounted for 41.05%, attache ciliates for 22.94%, free-swimming ciliates for 23.54% while crawling ciliates constituted only 12.47% of the dominant protozoa communities in the C-SBR bioreactor. The situation was different in the Cu-SBR bioreactor: after copper nanoparticles were added, the percentage of crawling ciliates increased significantly to 49.85%. However, the percentage of testate amoebae, free-swimming ciliates and attached cilitates decreased. The observed microorganisms are necessary to remove contaminants from wastewater. At lower sludge loads, protozoa are present in the activated sludge and account for several percent of the total biomass [27].

Protozoa are essential to reduce contaminants in wastewater. As long as the sludge load is not very high, protozoa are present in activated sludge and account for several percent of the total biomass. This is a characteristic feature of such sludge [26]. A significant presence of testate amoebae was observed in both reactors, but after the addition of CuNPs, their content decreased by about 16% in the Cu-SBR reactor. Their presence and the presence of ciliates shows that activated sludge was sufficiently oxygenated and mature.

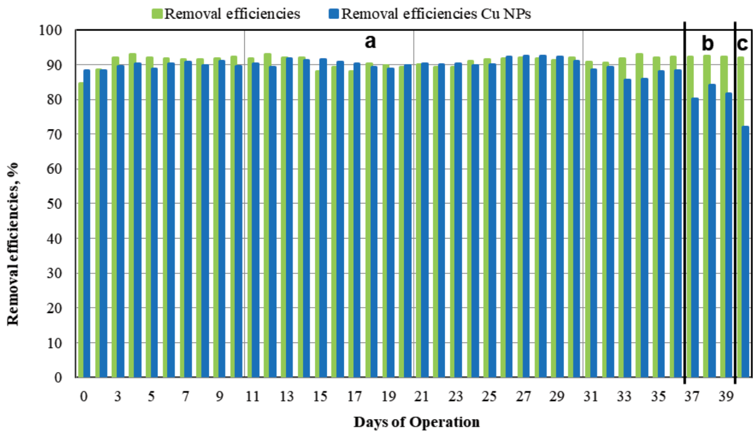

Fig. 2. Effects of copper nanoparticles on TOC removal efficiency in Cu-SBR and C-SBR reactors.

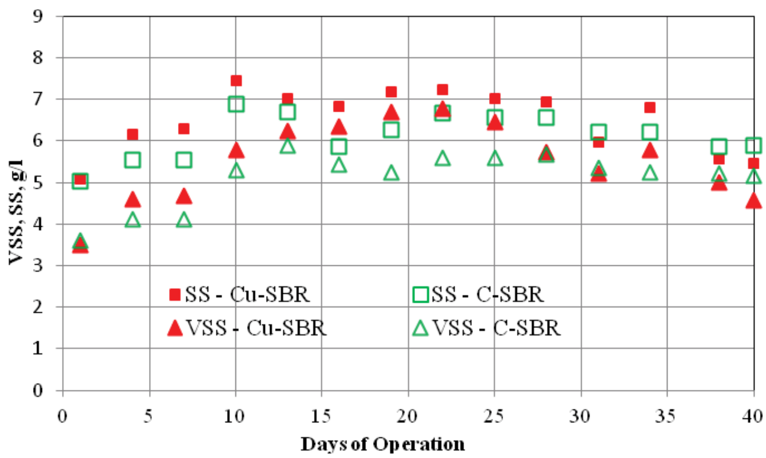

Fig. 3. Changes in SS and VSS during wastewater treatment in SBR reactors.

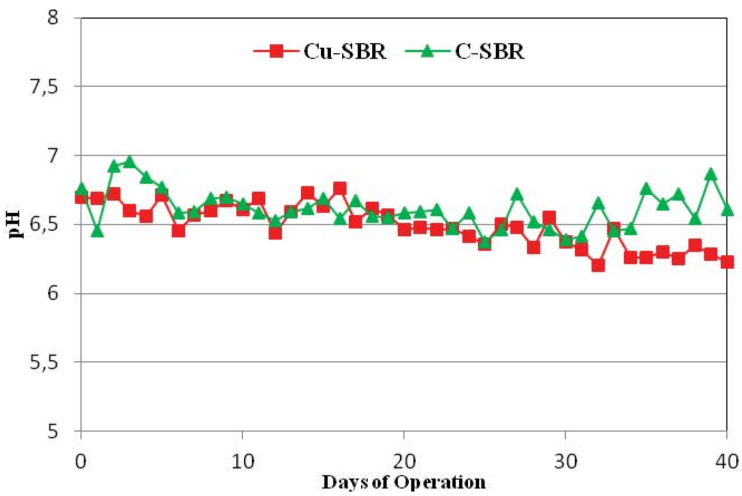

Fig. 4. Changes in pH during wastewater treatment in SBR reactors.

The study analyzed total copper concentration in the wastewater treated in both bioreactors. In the acclimatization phase, the analysis showed no copper in the treated wastewater. Fig. 6 presents the results from the phase after the addition of copper nanoparticles to the Cu-SBR bioreactor (from 30th to 40th day of the process). On the 31st day of the process after 3 mg/L of CuNPs was added to the Cu-SBR bioreactor, these nanoparticles showed a concentration of

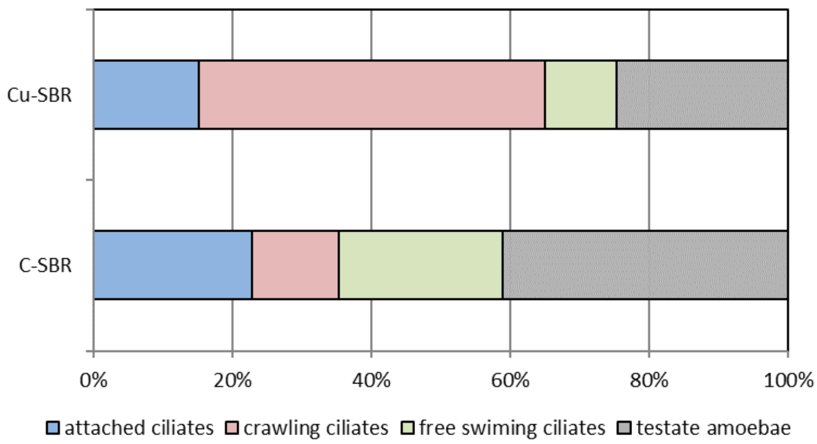

Fig. 5. Participation of dominant microorganisms in activated sludge in SBR bioreactors.

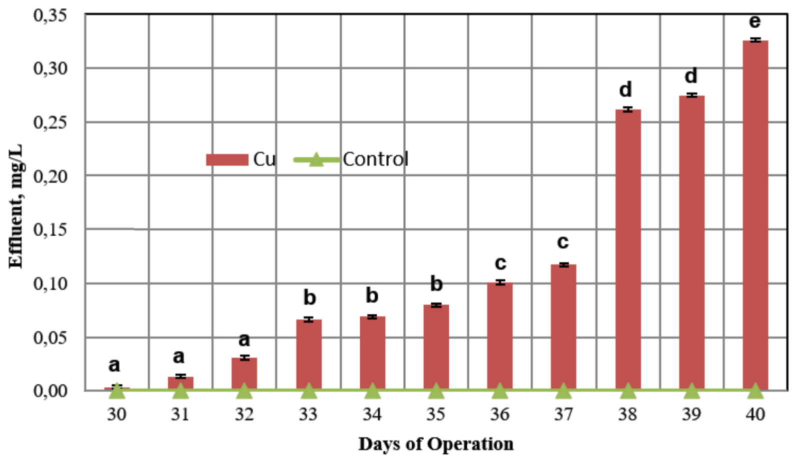

Fig. 6. Total Cu concentration in the SBR effluents.

0.0035 mg/L in the treated wastewater. After 3 d, the concentration of copper in the outflow increased by 80%, in the following days the concentration in the outflow increased very significantly to reach 90% after 10 d. This shows the poorer opportunities for microorganisms' removal of copper nanoparticles from wastewater. Whereas, Miao et al. [11] showed that the removal of copper at concentrations of 1 and 10 mg/L CuO NPs using a wastewater biofilm during 4 h of contact was respectively 51.4 and 42.5%, the author suggested that the nanoparticles were accumulated in the biofilm through biosorption. Zhang et al. [28] reported that long-term exposure to 5 mg Cu/L CuNPs reduced physiological activity and abundance of anammox bacteria, which resulted in deterioration of reactor performance. When studying in a SBR the performance had no evident change at 0–10 mg/L CuO NPs, while the CuO NPs concentration at 30–60 mg/L affected the COD removal rate and microbial enzymatic activity of activated sludge [24]. Zhang et al. [29] were carried out batch experiments at CuO NPs concentrations of 0, 1, 3, 5, 10, and 30 mg/L, respectively, found that the short-term exposure of CuO NPs performed no impact on the bioactivity of organisms. On the other hand, a long exposure of active sludge to nanoparticles had a bad influence on the bioactivity of microorganisms, especially at higher concentrations of NPs. On the other hand, long-term exposure to 5 mg Cu/L CuNPs reduced the activity and abundance of anammox bacteria, which resulted in reduced treatment efficiency [28].

4. Conclusions

The study analyzed the effect of CuNPs copper nanoparticles on the effectiveness of wastewater treatment in the SBR bioreactor. The concentration of nanoparticles equal to 3 mg/L CuNPs in wastewater did not significantly affect the removal of TOC in SBR. In the Cu-SBR bioreactor, the TOC removal rate dropped from 90.93% to 71.90% after a 10 d application of copper nanoparticles to wastewater.

CuNPs were effectively removed from wastewater and their small percentage was released to the environment through treated wastewater. The study showed that the concentration of copper in the treated wastewater increases strongly with the continuous supply of CuNP to the bioreactor. It was observed an almost 100-fold increase of copper concentration in the outflow from the Cu-SBR bioreactor. As the results showed, there is a threat to the environment from copper nanoparticles that get into the treated wastewater. Therefore, it should be presumed that they may pose a threat to the environment. The studies were conducted by Ganesh et al. [30] showed that the main removal paths these compounds were their aggregation, sedimentation and biosorption in the sediment. CuNPs nanoparticles may have a negative effect on the activity of activated sludge in SBR reactors. After addition of CuNPs, changes in the composition of the activated sludge microorganisms were observed, with the largest changes observed for the increase in the number of crawling ciliates and the decrease in the percentage of testate amoebae.

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