



# Nano- and microparticles-induced effect on activated sludge properties

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## Abstract

Nanomaterials have been usually perceived as a potential contamination of wastewater and thus tested in this context. On the contrary to this approach, here the possibility of the application of mineral microparticles or nanoparticles to improve the operation of activated sludge systems was studied for the first time. This work was aimed at checking the influence of aluminum oxide micro- and nanoparticles on morphology and settling properties of activated sludge. It was found that aluminum oxide micro- and nanoparticles changed the morphology of activated sludge flocs making them more circular. Moreover, the addition of aluminum oxide microparticles at the concentration up to 0.5 g l<sup>-1</sup> or aluminum oxide nanoparticles at the concentration up to 0.25 g l<sup>-1</sup> to activated sludge system caused the increase in flocs size. The changes in flocs morphology induced by aluminum oxide micro- or nanoparticles improved the separation processes in wastewater treatment systems and simultaneously did not deteriorate the efficiency of organic pollutants removal. It indicates the possibility to use aluminum oxide micro- or nanoparticles at the appropriate concentrations in wastewater treatment plants as a new solution to struggle against the bulking events.

**Keywords** Activated sludge · Aluminum oxide · Microparticles · Morphology · Nanoparticles · Separation

## Introduction

Nanomaterials due to their beneficial physical and chemical properties, particularly small size and large specific surface area, have many applications in different areas of industry. The wide range of applications increased the risks of their potential release into the environment (Qu et al. 2013; Santhosh et al. 2016). Thus, many types of nanomaterials, including zinc oxide (ZnO), aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), cerium (IV) oxide (CeO<sub>2</sub>), silicon (IV) oxide (SiO<sub>2</sub>) and silver (Ag), were tested toward their fate in wastewater treatment plants and/or their effect on biological treatment

processes. It was found that the presence of mineral nanoparticles (NPs) in wastewater usually did not significantly influence organic matter removal, nitrification and denitrification (Chen et al. 2012, 2014; Qiu et al. 2016; Wang et al. 2012) although in some conditions the decrease in nitrogen removal was observed (Chen et al. 2012; Cervantes-Avilés and Cuevas-Rodríguez 2017; Wang et al. 2012). Generally, the degrees of nitrogen and phosphorus removal decreased with the increase in NPs concentration (Chen et al. 2012; Wang et al. 2016; Li et al. 2017). Xiao et al. (2017) proposed the application of the magnetic mineral particles to remove and recover phosphorus from the secondary effluent of the wastewater treatment plant.

Regarding the fate of NPs in the conventional activated sludge systems, it was found that nanoparticles usually adsorbed onto activated sludge flocs and then they entered the interior of the microbial cells (Chen et al. 2012; Qiu et al. 2016; Cervantes-Avilés and Cuevas-Rodríguez 2017; Wang et al. 2016; Xiao et al. 2017). In this context, the question raised is whether the presence of nanomaterials in wastewater positively or negatively influenced the flocs morphology and settling properties. The studies performed so far have not answered this question. Limited data published in this

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field are contradictory. In the case of microparticles (MPs), hardly any data concerning their effect on activated sludge system have not been published yet. Regarding nanoparticles, Cervantes-Avilés and Cuevas-Rodríguez (2017) found that ZnO–NP improved the settling properties of activated sludge; however, the flocs size decreased or increased depending on the composition of wastewater (filtered or not-filtered) used in the experiments. At the same time, Qiu et al. (2016) observed that sludge volume index (SVI) increased due to the presence of silver nanoparticles. Ag NPs contributed to the overproduction of extracellular polymeric substances (EPS) and soluble microbial products (SMP) (Qiu et al. 2016), which deteriorated the sedimentation of suspended solids and induced turbidity in treated wastewater.

Taking into account that nanomaterials adsorb onto surface of activated sludge flocs and subsequently penetrate into the internal regions of these microbiological aggregates, it is highly probable that they change the morphology of flocs and exert the effect on their settleability.

Therefore, in this work the influence of mineral micro- and nanoparticles on morphology of activated sludge flocs and sludge settling properties was studied. It is the first attempt, in which effect of mineral ( $\text{Al}_2\text{O}_3$ ) MPs and NPs on activated sludge was compared. It was made with regard to the possible application of MPs and/or NPs for the enhancement of sedimentation processes.  $\text{Al}_2\text{O}_3$  MPs and NPs were selected due to their inert behavior, i.e., no changes of pH, no leachate products and no toxic effect toward microorganisms, in the cultivation of pure cultures of microorganisms (Etschmann et al. 2015). Some other mineral compounds, e.g.,  $\text{SiO}_2$ , iron (II, III) oxide, were not as inert as  $\text{Al}_2\text{O}_3$  (Etschmann et al. 2015). All experimental works were made at Lodz University of Technology (Poland) from June to August 2018.

## Materials and methods

### Size of nano- and microparticles tested

In this work, aluminum oxide nanoparticles ( $\text{Al}_2\text{O}_3$  NPs) and microparticles ( $\text{Al}_2\text{O}_3$  MPs) were selected to the tests.  $\text{Al}_2\text{O}_3$  NPs were purchased from Sigma-Aldrich (Sigma-Aldrich, Germany), while  $\text{Al}_2\text{O}_3$  MPs were obtained from Fluka (Fluka, USA). According to the manufacturers' data, the particle size of  $\text{Al}_2\text{O}_3$  NPs was less than 50 nm, whereas the particle size of  $\text{Al}_2\text{O}_3$  MPs was on average  $\leq 10 \mu\text{m}$ . The purity was 99.5%.

### Characterization of activated sludge and synthetic wastewater

Activated sludge was from the aeration part of the bioreactor operating in the Combined Wastewater Treatment Plant

(WWTP) in Lodz (Poland). The properties of activated sludge were as follows: The total suspended solids (TSS) were from 3.6 to 4.2  $\text{g l}^{-1}$ , and volatile suspended solids (VSS) were from 2.7 to 3.4  $\text{g l}^{-1}$ . Sludge volume index (SVI) ranged from 116 to 142  $\text{ml g TSS}^{-1}$ . Flocs can be classified as small on average (mean diameter from 77 to 88  $\mu\text{m}$ ) and irregular (circularity ranged from 0.317 to 0.379). The average number of filamentous bacteria corresponded to category 2 of the classification formulated by Eikelboom (2000).

The detailed composition of synthetic wastewater used in the tests was presented elsewhere (Gendaszewska and Liwarska-Bizukojc 2013).

### Activated sludge tests

The tests were aerobically carried out in shake flasks in the batch mode. First, the appropriate amount of NPs or MPs was weighted and carefully transferred into Erlenmeyer flask of the total volume of 350 ml. Then, 160 ml of fresh synthetic wastewater was added and finally 60 ml of activated sludge biomass was introduced to each Erlenmeyer flask. Activated sludge biomass concentration at the beginning of the tests was  $990 \pm 85 \text{ mg VSS l}^{-1}$ . At the same time, the following concentrations of NPs or MPs were tested: 0.25, 0.50, 1.00 and 2.00  $\text{g l}^{-1}$ .

Each of the experiments lasted for 24 h. They were performed at  $20 \pm 0.5 \text{ }^\circ\text{C}$  in a rotary shaker Certomat<sup>®</sup> IS at speed of  $130 \text{ min}^{-1}$ . Also the control tests (without addition of NPs or MPs) were performed according to the same procedure. All tests were made in triplicate.

### Analytical methods

At the start and at the end of each test, chemical oxygen demand (COD), TSS, VSS, SVI and turbidity were determined (APHA-AWWA-WEF 2012).

TSS and VSS were determined gravimetrically and expressed in  $\text{mg l}^{-1}$ . In order to determine TSS, a well-mixed sample of known volume was filtered and then the filter was dried at  $105 \text{ }^\circ\text{C}$ , cooled, desiccated and weighted until a constant weight was obtained. Next, the filter was placed into a porcelain crucible and combusted at  $550 \text{ }^\circ\text{C}$ , and then again cooled, desiccated, weighed until a constant weight of ash was obtained. VSS was calculated as the difference between TSS and ash.

Apart from the above-mentioned methods, digital image analysis of images was used for the purpose of the determination of the basic morphological descriptors of activated sludge flocs. The activated sludge suspension was mixed properly in the Erlenmeyer flask, and sampling was made with the use of polypropylene Pasteur pipette possessing a tip of diameter equal to 3 mm that was ideal for the preparation of slides from inhomogeneous suspension. As a result,



four vital unstained slides of activated sludge sample were prepared. A light microscope Nikon Eclipse Ni was used for bright-field observations. The magnification of the objective lens was  $4\times$ . From each sample, not less than 40 RGB images were snapped, processed and analyzed by the automated procedure elaborated in NIS-Elements AR software (Nikon, Japan). The following morphological parameters of the flocs were measured: projected area, perimeter, equivalent diameter, convexity and circularity. The definitions of these parameters and image analysis procedure were presented elsewhere (Gendaszewska and Liwarska-Bizukojc 2013).

### Calculation and statistical elaboration of the results

The statistical elaboration of image analysis data was performed using MS Excel. It comprised the calculation of mean values, standard deviation ( $\sigma$ ) and the tests of goodness of fit for different models of distribution (e.g., normal, log-normal Lorentz and Voigt) of activated sludge flocs diameters.

The relative change in flocs area ( $A_C$ ) was calculated to quantify the increase or decrease in flocs size. It was calculated according to the following equation:

$$A_C = \frac{(A_{24} - A_0)}{A_0} \cdot 100\% \quad (1)$$

where  $A_{24}$  is the mean projected area of the flocs after 24 h of the test and  $A_0$  is the mean projected area of the flocs at the beginning of the tests.

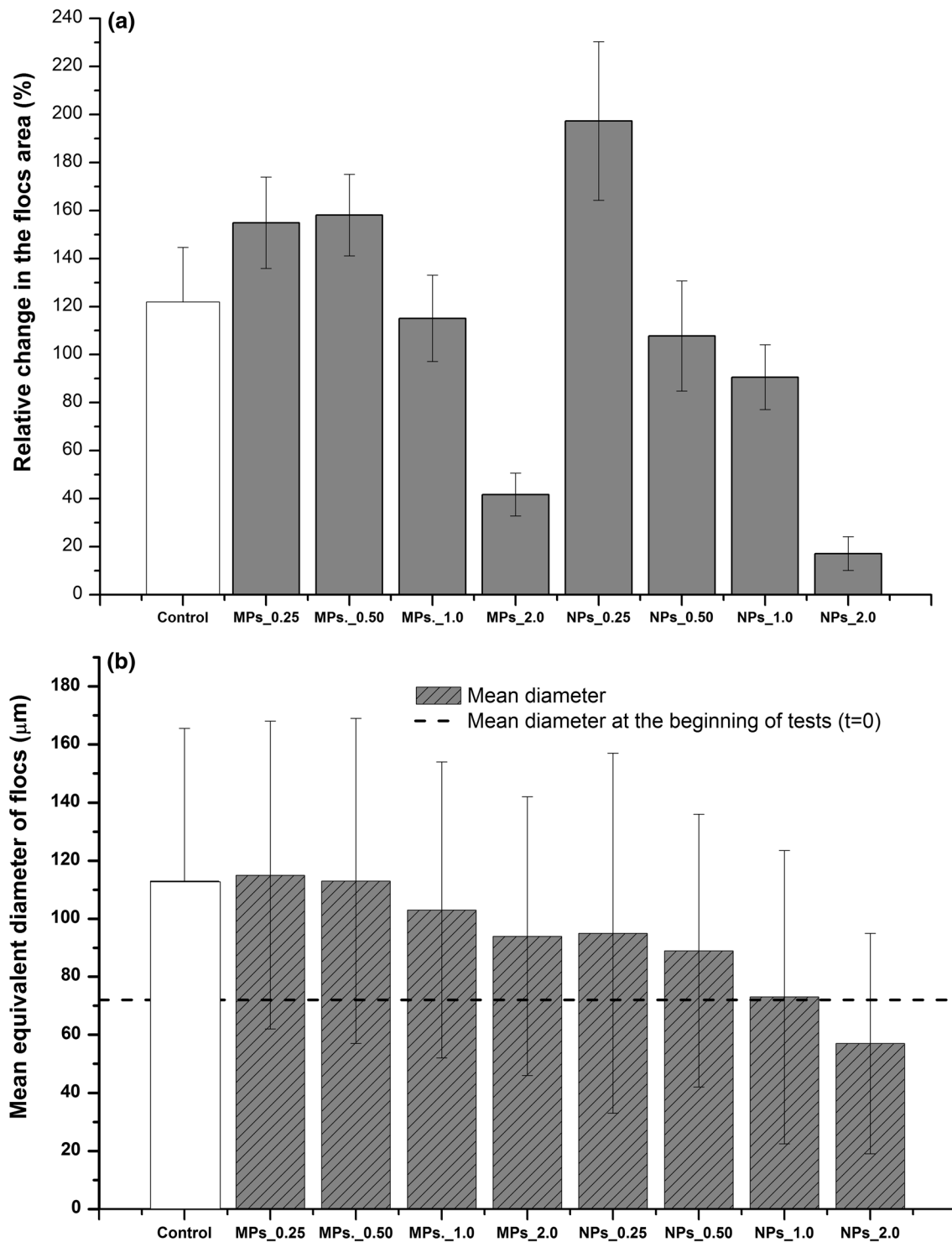
### Results and discussion

Generally, the size of activated sludge flocs increased for the duration of the experiments. It was confirmed by all morphological parameters measured, particularly by the mean projected area of flocs. The relative change in flocs area was 122% in the control run, whereas the addition of  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs contributed to the higher increase in activated sludge flocs (from 155 to 197%) than it was observed in the control run. However, it was found only at lower concentrations of micro- or nanoparticles added (Fig. 1a). In the case of  $\text{Al}_2\text{O}_3$  MPs, it was observed at  $0.25 \text{ g l}^{-1}$  and  $0.5 \text{ g l}^{-1}$ , while in the case of  $\text{Al}_2\text{O}_3$  NPs, only at  $0.25 \text{ g l}^{-1}$ . At higher concentrations of  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs tested (above  $0.5 \text{ g l}^{-1}$ ), the relative change in flocs area was lower than in the control (Fig. 1a). It is consistent with the observations made by Cervantes-Avilés and Cuevas-Rodríguez (2017). They showed that the size of activated sludge flocs was gradually decreasing when the concentration of ZnO NPs

increased from  $450$  to  $2000 \text{ mg l}^{-1}$  in the experiments with raw (not-filtered) wastewater.

The mean equivalent diameters of flocs also increased in the course of the test, excluding the run with the highest concentration of  $\text{Al}_2\text{O}_3$  NPs ( $2 \text{ g l}^{-1}$ ) (Fig. 1b). The value of mean equivalent diameter of flocs in the control was equal or higher compared to the values of mean equivalent diameter of flocs in the runs with  $\text{Al}_2\text{O}_3$  MPs or NPs. The effect of  $\text{Al}_2\text{O}_3$  MPs and  $\text{Al}_2\text{O}_3$  NPs on the diameter of activated sludge flocs was different. The addition of  $\text{Al}_2\text{O}_3$  MPs contributed to the increase in the equivalent diameter of flocs to the higher extent than it was observed for  $\text{Al}_2\text{O}_3$  NPs. As a result, the mean equivalent diameters of flocs in the runs with  $\text{Al}_2\text{O}_3$  MPs were higher than those in the runs with  $\text{Al}_2\text{O}_3$  NPs. For example, at the lowest of concentrations studied ( $0.25 \text{ g l}^{-1}$ ), the mean floc equivalent diameter was  $115 \mu\text{m}$  in the runs with  $\text{Al}_2\text{O}_3$  MPs, while in the runs with  $\text{Al}_2\text{O}_3$  NPs, it was  $95 \mu\text{m}$ . Moreover, the mean equivalent diameter of flocs decreased with the increase in  $\text{Al}_2\text{O}_3$  MPs or NPs concentrations. Similar phenomenon was also observed for the mean projected area, particularly in the case of  $\text{Al}_2\text{O}_3$  NPs (Fig. 1a). The values of mean equivalent diameters and mean projected areas measured in this study indicated that the addition of  $\text{Al}_2\text{O}_3$  MPs at the concentrations above  $0.5 \text{ g l}^{-1}$  or the addition of  $\text{Al}_2\text{O}_3$  NPs at the concentration above  $0.25 \text{ g l}^{-1}$  contributed to the domination of smaller flocs than it was observed in the activated sludge system without the addition of  $\text{Al}_2\text{O}_3$  MPs or NPs (Fig. 1). The decrease in flocs size caused by the addition of  $\text{Al}_2\text{O}_3$  MPs or NPs was most probably the result of the destruction of microbial agglomerates, namely activated sludge flocs. Cervantes-Avilés and Cuevas-Rodríguez (2017) also observed this phenomenon in the case of raw wastewater and explained it similarly as Hou et al. (2015) by lower flocculation ability caused by the overproduction of loosely bound EPS. In the case of pure cultures of filamentous fungi, it was proved that the mineral MPs usually contributed to the decrease in agglomerates diameter. They also loosened their structure (Krull et al. 2013). However, mineral MPs as well NPs may also enhance the agglomeration processes. It was shown by Cervantes-Avilés and Cuevas-Rodríguez (2017) in the tests with filtered wastewater, when the presence of ZnO NPs favored the formation of larger flocs than those in the control test. Kowalska et al. (2018) observed that  $\text{Al}_2\text{O}_3$  MPs can accelerate the agglomeration of spores and small mycelial objects. The results reported in literature as well as these obtained in this work indicated that the effect of mineral MPs or NPs on the morphology of microbial agglomerates may be different and depends on the concentration, type and the size of particles (MPs or NPs) added, the initial morphology and physiological state of the microbial agglomerates and composition of substrate.





**Fig. 1** Effect of  $\text{Al}_2\text{O}_3$  MPs and NPs on the size of activated sludge flocs: **a** the relative change in the flocs area with the standard deviations; **b** the mean equivalent diameter of flocs with the standard deviations

The distributions of equivalent diameters of flocs corresponded to the log-normal distribution in the case of each test, i.e., with and without  $\text{Al}_2\text{O}_3$  MPs or NPs (Fig. S1). In order to describe them, the following equation was applied:

$$y = y_o + \frac{A}{\sqrt{2 \cdot \pi \cdot \sigma \cdot x}} \cdot \exp \frac{-\left(\ln \frac{x}{\mu}\right)^2}{2 \cdot \sigma^2} \quad (2)$$



where  $x$  is flocs equivalent diameter,  $\mu$  is the mean value of flocs equivalent diameter,  $\sigma$  is the standard deviation,  $y_0$  is the offset frequency and  $A$  is the area under the curve within limits  $\pm \sigma$ .

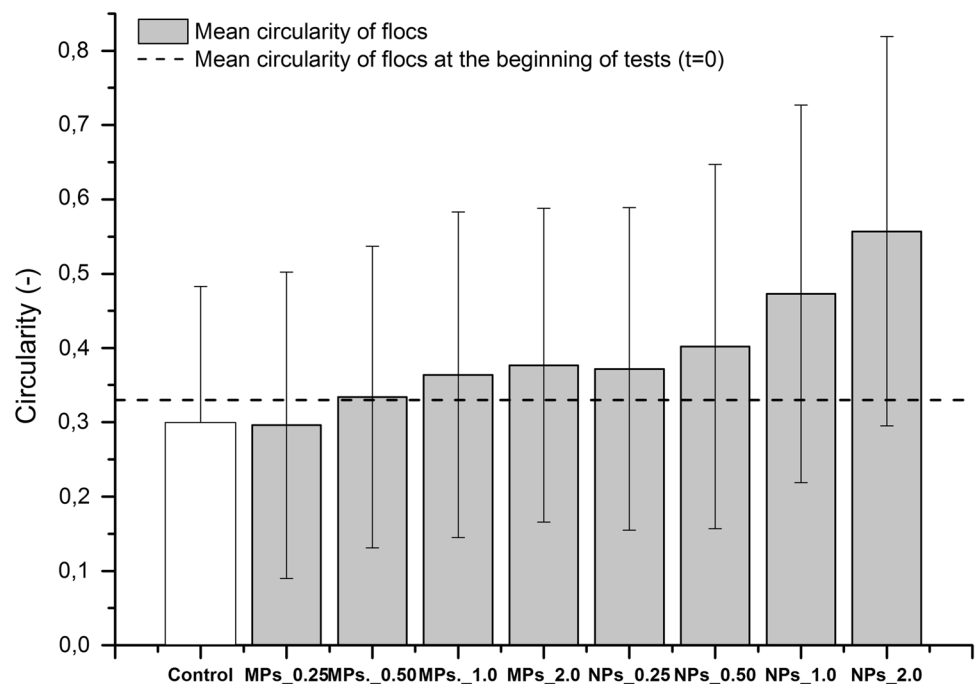
The correlation coefficients  $R^2$  ranged from 0.717 to 0.929. The log-normal distribution is frequently observed in various biological systems including activated sludge systems as it was previously shown (Wilén and Balmér 1999). In each test, irrespective of the presence and concentration of  $\text{Al}_2\text{O}_3$  micro- and nanoparticles, the most frequent were flocs of diameter from 20 to 30  $\mu\text{m}$ .

The addition of  $\text{Al}_2\text{O}_3$  MPs or NPs also influenced the circularity of the activated sludge flocs. The higher the concentration of  $\text{Al}_2\text{O}_3$  MPs or NPs was, the more circular the formed flocs were. The  $\text{Al}_2\text{O}_3$  NPs acted stronger on the circularity of flocs than  $\text{Al}_2\text{O}_3$  MPs. As a result, the flocs were more circular in the runs with  $\text{Al}_2\text{O}_3$  NPs than with  $\text{Al}_2\text{O}_3$  MPs (Fig. 2). In any run, the mean circularity did not exceed 0.560, indicating that the shape of flocs was relatively far from the ideal circle (circularity equal to 1). Simultaneously, the values of mean circularity obtained in the runs with  $\text{Al}_2\text{O}_3$  NPs (at concentration not lower than 0.5  $\text{g l}^{-1}$ ) were higher than the values of mean circularity observed during 1-year monitoring of flocs from two wastewater treatment plants (Liwarska-Bizukojc et al. 2015), which usually were below 0.410. The stronger impact of  $\text{Al}_2\text{O}_3$  NPs than  $\text{Al}_2\text{O}_3$  MPs on flocs circularity was most probably connected with the size of particles added. Smaller particles changed the morphology of activated sludge flocs to the higher extent contributing to the destruction of agglomerates and, as a result, to the decrease in the diameters of flocs. Thus, the

flocs were lower in the experiments with  $\text{Al}_2\text{O}_3$  NPs than those in the experiments with  $\text{Al}_2\text{O}_3$  MPs (Fig. 1b). The formation of smaller flocs is often accompanied by their higher circularity in activated sludge systems (Amaral and Ferreira 2005; Bitton 2005). The effect of micro- and nanoparticles on the activated sludge flocs, particularly on their shape, is also seen in the microscopic images shown in Fig. 3.

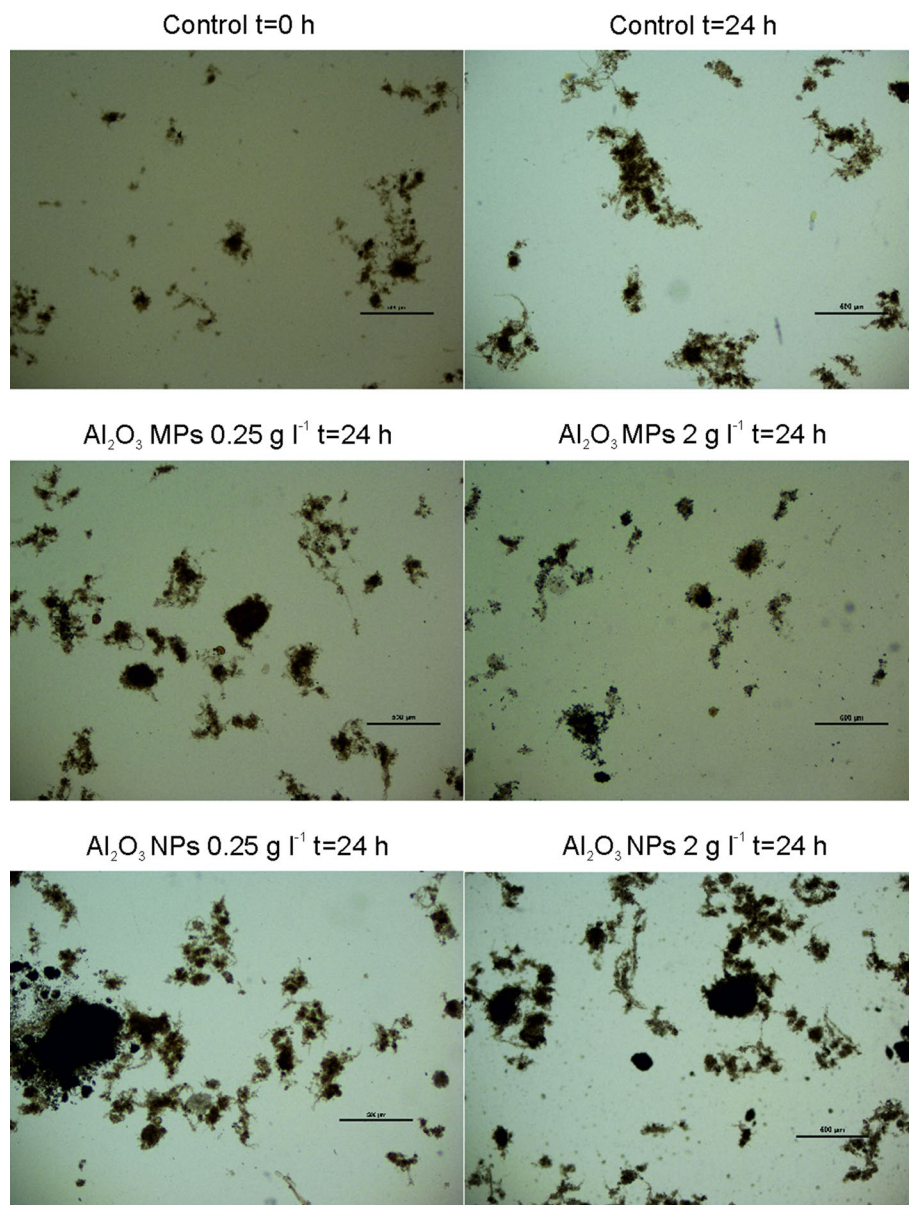
The changes in flocs morphology induced by the addition of  $\text{Al}_2\text{O}_3$  MPs or NPs to the activated sludge system influenced the settling properties of sludge. The presence of micro- or nanoparticles of  $\text{Al}_2\text{O}_3$  improved the settling ability of activated sludge. It was confirmed by the values of SVI, which were lower in the processes with  $\text{Al}_2\text{O}_3$  MPs or NPs compared to the control runs (Fig. 4). SVI decreased with the increase in the concentration of  $\text{Al}_2\text{O}_3$  MPs or NPs. It was most probably connected with the increase in flocs density due to the adsorption of mineral particles onto activated sludge flocs, and it was previously observed by Cervantes-Avilés and Cuevas-Rodríguez (2017) too. Additionally, the increase in flocs circularity favored the improvement in flocs settling properties as it could have been expected on the basis of literature data (Eikelboom 2000). The settling velocity of the flocs is reduced if they are irregularly shaped (Eikelboom 2000). The flow resistance is higher for the settling objects of irregular shape than for the circular objects. What is interesting, the decrease in flocs size at higher concentrations (1 and 2  $\text{g l}^{-1}$ ) of  $\text{Al}_2\text{O}_3$  MPs or NPs contributed neither to the deterioration of the sedimentation processes, nor to the appearance of turbidity of treated wastewater. It was most probably due to two reasons. First, the decrease in flocs size was not significant,

**Fig. 2** Effect of  $\text{Al}_2\text{O}_3$  MPs and NPs on the circularity of activated sludge flocs; the bars reflect the standard deviation of the circularity





**Fig. 3** Comparison of morphology of activated sludge flocs exposed and not exposed (control) to  $\text{Al}_2\text{O}_3$  micro- and nanoparticles



particularly in comparison with the beginning of the experiment. Second, as it was proved by microscopic observations,  $\text{Al}_2\text{O}_3$  micro- or nanoparticles were incorporated into sludge flocs increasing their specific gravity. Similar phenomenon was found earlier in the case of other nanomaterials such as  $\text{SiO}_2$  NPs,  $\text{ZnO}$  NPs,  $\text{Ag}$  NPs or  $\text{TiO}_2$  NPs (Qiu et al. 2016; Cervantes-Avilés and Cuevas-Rodríguez 2017; Wang et al. 2016; Li et al. 2017).

The presence of  $\text{Al}_2\text{O}_3$  MPs or NPs in wastewater at the concentrations up to  $2 \text{ g l}^{-1}$  did not deteriorate the efficiency of biological treatment processes. The degree of COD removal was at the level from 74 to 80%. It was similar as in the control run, in which its mean value was  $78\% \pm 2$ .

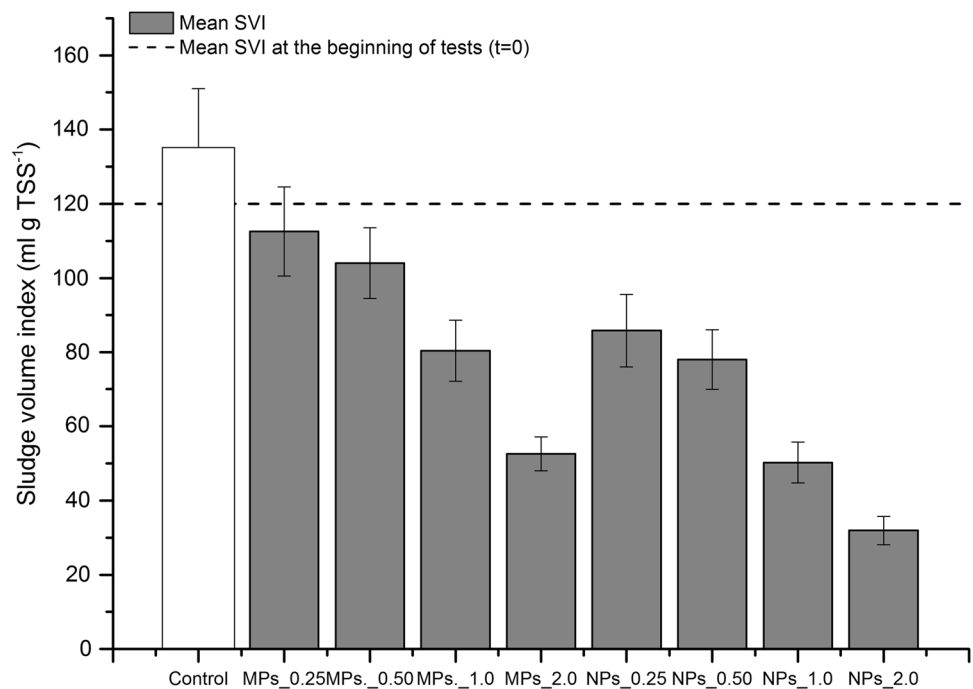
The application of mineral particles in activated sludge systems has benefits and drawbacks. It depends on chemical

composition and concentration of the MPs or NPs used. Upon the own experiments presented in this work and literature data, the advantages and disadvantages of the use of mineral micro- or nanomaterials in activated sludge processes are listed down in Table 1.

## Conclusion

1.  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs change the shape of flocs into more circular form. Effect of  $\text{Al}_2\text{O}_3$  NPs on the circularity of flocs is stronger than that induced by  $\text{Al}_2\text{O}_3$  MPs.
2. The size of activated sludge flocs depends on the concentration of  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs added and the size of particles (MPs or NPs). The addition of  $\text{Al}_2\text{O}_3$

**Fig. 4** Effect of  $\text{Al}_2\text{O}_3$  MPs and NPs on settling properties of activated sludge; the bars reflect the standard deviation of SVI



**Table 1** Benefits and drawbacks of the use of mineral MPs or NPs in activated sludge systems

Benefits	Drawbacks
Improvement in settling properties of activated sludge (this work; Cervantes-Avilés and Cuevas-Rodríguez 2017)	The presence of additional contamination, i.e., mineral particles, in the sludge (particularly in the excess sludge removed from the system)
Negligible effect or no deterioration of the removal of organic compounds expressed as COD from wastewater (this work; Chen et al. 2012; Wang et al. 2012; and Qiu et al. 2016)	Decrease in nitrogen removal from wastewater (Chen et al. 2012; Cervantes-Avilés and Cuevas-Rodríguez 2017; and Wang et al. 2016)
No effect or enhancement of phosphorus removal from wastewater (Cervantes-Avilés and Cuevas-Rodríguez 2017; Xiao et al. 2017)	Decrease in the activity of activated sludge microorganisms (Cervantes-Avilés et al. 2017; Wang et al. 2016)

MPs at the concentration up to  $0.5 \text{ g l}^{-1}$  or  $\text{Al}_2\text{O}_3$  NPs at the concentration up to  $0.25 \text{ g l}^{-1}$  to activated sludge system contributes to the increase in flocs size, while higher concentrations of  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs contribute to the destruction of flocs and decrease in their size.

- The changes in morphology of flocs induced by  $\text{Al}_2\text{O}_3$  MPs or  $\text{Al}_2\text{O}_3$  NPs act positively on settling properties of activated sludge and simultaneously do not deteriorate the efficiency of organic pollutants removal.

Summing up, the addition of  $\text{Al}_2\text{O}_3$  MPs or NPs at the appropriate concentrations improves the efficiency of separation processes in the wastewater treatment systems and facilitates the sludge management.

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