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Effect of aluminium oxide nano- and microparticles on the functional groups of microorganisms of activated sludge

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ABSTRACT

In this work the impact of aluminium oxide nanoparticles (Al_2O_3 NPs) and aluminium oxide microparticles (Al_2O_3 MPs) on three functional groups of activated sludge microorganisms, that is, heterotrophic bacteria (HET), ammonium oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), was studied. In order to describe it quantitatively, the degree of inhibition was calculated. Additionally, the values of Monod kinetic parameters and stoichiometric yield coefficients (Y_{HET}) were determined for HET. Neither the biochemical activity nor specific growth rate of HET was inhibited by Al_2O_3 NPs or MPs in the activated sludge system. The degree of inhibition of the biochemical activity of HET did not usually exceed 20%. At the same time, the biochemical activity of nitrifying microorganisms was significantly inhibited by both Al_2O_3 NPs and MPs that was confirmed by the values of degrees of inhibition in the range from 42.6% to 84.3%. Out of the nitrifying microorganisms, the AOB were affected stronger than NOB. Al_2O_3 NPs inhibited the biochemical activity of HET and nitrifying microorganisms, particularly AOB, to a higher extent than Al_2O_3 MPs did. The lower values of Y_{HET} in the tests with Al_2O_3 NPs or MPs than in those without their addition indicated that the presence of Al_2O_3 NPs or MPs contributed to the decrease of the activated sludge biomass concentration.

Keywords: Biochemical activity; Heterotrophic bacteria; Inhibition; Kinetics of growth; Nitrifiers

1. Introduction

The interest and application of mineral nano- and micromaterials in different areas of industry is still increasing. In parallel, these materials are extensively studied towards their fate and behavior in the environment as well as their effect on aquatic and terrestrial organisms. Due to the fact that wastewater treatment plants (WWTPs) are believed to be the main route of the release of nanoparticles (NPs) and microparticles (MPs) to the environment, many studies have focused on the impact of these particles on activated sludge systems.

A variety of engineered nanoparticles have been studied so far. These were metals (e.g. Ag, Cu, Fe), metal oxides (e.g. TiO_2, ZnO, Al_2O_3), carbon compounds (e.g. graphene, fullerene) and other ones (e.g. silica, quantum dots) [1]. On one hand mineral, NPs/MPs were perceived as pollutants that might have an impact on activated sludge microorganisms and, as a consequence, on the efficiency of wastewater treatment processes. On the other hand, some of them (CeO_2 NPs, magnetite mineral MPs, keramsite MPs) were intentionally added to activated sludge systems in order to improve their operation, for example, sludge settleability, heavy metals or organic pollutants removal [2–7].

With regard to the microparticles, their effect on activated sludge systems has not been thoroughly studied so far, as it was made in the case of NPs. The previous studies focused on the intentional application of microparticles

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in activated sludge systems aiming at the improvement of their operation and efficiency. For example, Xiao et al. [6] tested the applicability and chemical stability of magnetite mineral microparticles of average particle size equal to 34 μm for phosphorus removal and recovery from the secondary effluent of WWTPs. At the same time, Maslon et al. [7] checked, whether the efficiency of activated sludge systems increased, if microparticles of keramsite (predominate fractions below 50 μm) were added.

The studies concerning the influence of engineered NPs on activated sludge systems, particularly on the removal of carbon, nitrogen and phosphorus compounds, revealed that it depended on the chemical composition and concentration of NPs tested. At low concentrations of NPs in wastewater, that is, below 10 mg L^{-1}, the influence of NPs on the efficiency of COD, nitrogen and phosphorus removal was usually negligible [8–13]. It was observed for the nanomaterials of different chemical composition. Wang et al. [9] compared the effect of silver nanoparticles (Ag NPs), titanium dioxide nanoparticles (TiO_2 NPs), fullerene nanoparticles (C_{60} NPs) on the removal of organic compounds expressed as COD. It occurred that in the range of concentrations from 0.5 to 2.5 mg L^{-1} the effect of the abovementioned nanomaterials on the biodegradation of organic matter in an activated sludge system was negligible [9]. Qiu et al. [11] found that Ag NPs at concentrations up to 5 mg L^{-1} did not significantly influence either organic matter removal or nitrification or denitrification processes of wastewater treatment in the sequencing batch reactor (SBR). It was confirmed by Zhou and Xu [13], who showed that the addition of Ag NPs to wastewater at concentrations from 0.1 to 10 mg L^{-1} exhibited no significant effect on COD, nitrogen and phosphorus removal.

It was found that zinc oxide nanoparticles (ZnO NPs) at concentrations up to 5 mg L^{-1} had no effect on nutrients removal from wastewater [10]. However, the addition of ZnO NPs at concentrations from 10 to 60 mg L^{-1} decreased the removal of COD, nitrogen and phosphorus [10]. At the same time in the other work, the deterioration of efficiency of nutrients removal even at the concentration of ZnO NPs equal to 1 mg L^{-1} was observed [14]. These contradictive results were found although in both cases wastewater treatment processes were performed in the similar conditions in the same type of bioreactor, that is, sequencing batch reactor (SBR). Cervantes-Avilés et al. [15] observed the decrease of ammonium nitrogen removal from wastewater for all concentrations of ZnO NPs from 450 to 2,000 mg L^{-1}. Simultaneously, the addition of ZnO NPs at the same doses contributed to the improvement of phosphorus removal [15].

Regarding aluminium oxide nanoparticles (Al_2O_3 NPs) one of few works was published by Chen et al. [16], who showed that Al_2O_3 NPs at the concentrations from 1 to 50 mg L^{-1} induced a marginal influence on the efficiency of nitrification, denitrification and phosphorus removal during the short-term exposure experiments. However, the prolonged exposure of activated sludge biomass to Al_2O_3 NPs at the concentration of 50 mg L^{-1} caused to the decrease of total nitrogen removal from 80.4% to 62.5% but biological phosphorus removal was not affected.

Due to the shortage of data concerning the influence of aluminium oxide nanoparticles (Al_2O_3 NPs) and aluminium oxide microparticles (Al_2O_3 MPs) on activated sludge biomass these mineral materials were selected to be studied in this work. Simultaneously, it is worth noticing that Al_2O_3 NPs are considered as one of the most common nanomaterials entering the WWTPs [17]. Lazareva et al. [17] elaborated an environmental release model that allowed for the prediction of NPs concentration in the effluent and biosolids removed from a WWTPs. The model was formulated upon the global production of NPs calculated with the use of market production estimates of nanomaterials excluding carbon black. The predicted masses of NPs entering the WWTPs (expressed as g $person^{-1}$ $year^{-1}$) were the highest for the following nanomaterials: TiO_2, ZnO, Fe+FeO_x, Al_2O_3 and SiO_2 [17]. Some of them (e.g. ZnO) were thoroughly investigated with regard to their effect on activated sludge systems, whereas other ones (e.g. Al_2O_3) were hardly tested. At the same time, the predicted mass of Al_2O_3 NPs entering the WWTPs was between 0.82 and 3.69 g $person^{-1}$ y^{-1} [17].

The aim of this work was to describe quantitatively the influence of aluminium oxide nanoparticles (Al_2O_3 NPs) and aluminium oxide microparticles (Al_2O_3 MPs) on three functional groups of activated sludge microorganisms, that is, heterotrophic bacteria (HET), ammonium oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The description comprises the calculation of the degrees of inhibition for each group of microorganisms studied and the determination of the values of kinetic and stoichiometric parameters used in the activated sludge models for heterotrophic bacteria. It is one of the first studies, in which the effects of mineral nano- and microparticles on biochemical activity of activated sludge microorganisms were compared.

2. Materials and methods

2.1. Aluminium oxide particles

Al_2O_3 NPs were purchased from Sigma Aldrich (Germany), while Al_2O_3 MPs was obtained from Fluka (USA). According to the manufacturer data, the particle size of Al_2O_3 NPs was less than 50 μm, whereas the particle size of Al_2O_3 MPs did not exceed on average 10 mm. The purity of both materials was at the level of 99.5%.

2.2. Oxygen uptake rate tests

The metabolic activity of different functional groups of activated sludge microorganisms and the values of growth kinetic parameters of HET were determined with the use of two OUR tests of different methodology. In the first type of tests (subsection 2.2.1) the changes of dissolved oxygen (DO) concentration in time was only recorded, whereas in the second type of tests (subsection 2.2.2) the changes of OUR in time were recorded so as to determine the kinetic parameters.

2.2.1. Evaluation of biochemical activity of AOB, NOB, and HET

In order to evaluate the biochemical activity of AOB, NOB, and HET the first type of OUR tests were conducted. They were made according to the procedure described in [18]. The methodology of this test was based upon the determination of $N–NH_4^+$ and $N–NO_2^-$ oxidation rates by measuring the OUR of activated sludge samples before and after addition of the selective nitrification inhibitors. Apart from the tested compound, in this case, Al_2O_3 NPs or MPs, no external substrate was added. The concentration of Al_2O_3 NPs or MPs in these tests varied from 50 to 1,000 mg L^{-1}. This range of concentrations was selected taking the results obtained for Al_2O_3 NPs by Chen et al. [16] into account. Each test was performed in a cylindrical, tightly closed vessel of working volume equal to 100 ml. The vessel was equipped with the optical DO meter ProODO™ (YSI Environmental, USA) that was connected with the computer to register the changes of DO concentration and temperature. The tests ran at ambient temperature 22°C ± 1°C and agitation was assured by a magnetic stirrer. Activated sludge was taken from the aeration chamber of the Combined Wastewater Treatment Plant in Lodz (Poland) operating in University of Cape Town mode. Activated sludge had typical properties, that is, the content of total suspended solids (TSS) and volatile suspended solids (VSS) were 3.6 ± 0.2 and 2.1 ± 0.1 g L^{-1}, respectively. Sludge volume index (SVI) was at the level of 112 ± 8 ml g TSS^{-1}. Soluble COD and ammonium nitrogen were at the levels of 46 ± 5 mg O_2 L^{-1} and 1.27 ± 0.22 mg N L^{-1}, respectively. Al_2O_3 NPs or MPs were mixed with activated sludge biomass at the appropriate concentration and then the suspension was transferred to the reaction vessel. It was saturated with oxygen by the intensive agitation and then the agitation speed was kept constant at 150 rpm, and the consumption of DO was observed.

First, the total OUR was determined. No sooner had DO concentration decreased to approximately 3 mg O_2 L^{-1}, than sodium chlorate ($NaClO_3$), a selective inhibitor of nitrites oxidation, was added to inhibit NOB [18]. The difference between the total OUR and the OUR measured in the presence of $NaClO_3$ was regarded as the oxygen uptake necessary for nitrites oxidation, that is, associated with NOB activity. Next, a selective inhibitor of ammonium, nitrogen oxidation, that is, allylthiourea (ATU), was added to the reaction vessel in order to inhibit AOB [18]. The difference between the OUR measured after the addition of $NaClO_3$ and the OUR measured in the presence of both inhibitors ($NaClO_3$, ATU) represented the oxygen uptake necessary for ammonium nitrogen oxidation corresponding with the activity of AOB. The OUR for HET was measured as the remaining oxygen uptake after the addition of both selective inhibitors ($NaClO_3$ and ATU). At the same time, the OUR for all nitrifying microorganisms (together AOB and NOB) was calculated as the difference between the total OUR and OUR measured for heterotrophs. The selective inhibitors were freshly prepared stock solutions of ATU (125 mg L^{-1}) and $NaClO_3$ (0.5 M) [18].

Each time, the OUR was determined as the slope of the linear part of the DO concentration curve and expressed in mg O_2 L^{-1} h^{-1}. Then, it was related to VSS and finally, the activity of each functional group of activated sludge biomass was expressed as mg O_2 g VSS^{-1} h^{-1}. The OUR test for each concentration of Al_2O_3 NPs or MPs was made in triplicate. It also concerned the control OUR tests without the addition of Al_2O_3 NPs or MPs to activated sludge microorganisms that were performed in parallel. The difference between the biochemical activity of the selected group of microorganisms without the addition of Al_2O_3 NPs or MPs (A_0) and with the addition of Al_2O_3 NPs or MPs ($A_{NP/MP}$) related to the biochemical activity of the selected group of microorganisms without the addition of Al_2O_3 NPs or MPs (A_0) multiplied by 100% was defined as the degree of inhibition (%).

2.2.2. Determination of the kinetic parameters and stoichiometric coefficient for HET

For the determination of the kinetic parameters for the growth of HET taking part in the biological treatment of wastewater, the second type of OUR tests in a 4 L working volume batch bioreactor equipped with the control and measurement devices were performed. The tests were carried out at a constant temperature of 20°C. The methodology of these OUR tests was described elsewhere [19]. The tests were performed with the addition of Al_2O_3 NPs or MPs or without the addition of these particles (control tests). The concentration of Al_2O_3 NPs or MPs was equal to 250 mg L^{-1}. Each of the tests, including the control runs, was performed in triplicate. Firstly, synthetic municipal wastewater of the model composition: 300 mg peptone, 100 mg sodium acetate, 50 mg potassium monophosphate, 50 mg sodium hydrocarbonate, 50 mg ammonium hydrophosphate, 5 mg magnesium sulphate and 5 mg sodium chloride per litre [19] was introduced into the bioreactor. Chemical oxygen demand (COD) was at the level of 420 ± 5 mg L^{-1}, while total Kjeldahl nitrogen (TKN) was 49.5 ± 1.2 mg L^{-1}. Then, activated sludge suspension was mixed with the appropriate amount of Al_2O_3 NPs or MPs and added to the bioreactor. ATU was added in order to inhibit nitrification. After that DO and OUR was immediately measured with an optical DO meter ProODO™ (YSI Environmental, USA) and controlled with the help of software (Bioreactor 1.0; © Jakub Mielczarek, 2010). The biomass used in all OUR tests came from the laboratory activated sludge system and was acclimated to the synthetic municipal wastewater that was used in the OUR tests as it was described in [19].

On the basis of the changes of OUR in time (Fig. S1), the maximum specific growth rate of HET ($\mu_{max,HET}$) and the half-saturation constant (K_S) were determined. The values of $\mu_{max,HET}$ were calculated as it was described in [20], whereas the value of K_S was calculated according to the method proposed in [19]. Yield coefficient for the ordinary heterotrophs (Y_{HET}) was calculated as the ratio of biomass concentration change (expressed as VSS) to substrate concentration change (expressed as COD soluble). VSS and COD were determined at the beginning and at the end of OUR tests [19].

2.3. Physicochemical analysis

The following physicochemical analyses were made: COD, TSS, VSS, SVI, TKN and ammonium nitrogen. They were performed in agreement with the standard methodologies [21].

2.4. Statistical elaboration of the results

The basic statistical elaboration of results of the tests performed including the calculation of mean values and standard deviation (σ) was made with the use of MS Excel. One-way analysis of variance (ANOVA) was applied to estimate if the values of the biochemical activity of the appropriate group of microorganisms exposed to Al_2O_3 NPs or MPs and the biochemical activity of the appropriate group of microorganisms not exposed to Al_2O_3 NPs or MPs (control tests) were statistically equal. The null hypothesis stating that they were equal was assumed. ANOVA implemented in MS Excel (Analysis ToolPak) software was used. The confidence level of 95% was assumed. Kinetic parameters ($\mu_{max,HET}$, K_S) were determined with the help of OriginPro 9.0 (OriginLab Corporation, USA).

3. Results and discussion

3.1. Effect of Al_2O_3 NPs and MPs on the activity of AOB, NOB, and HET

The presence of Al_2O_3 NPs or MPs in the activated sludge system at the concentration of 50 mg L^{-1} or higher caused to the decrease of the biochemical activity of each functional group of microorganisms studied. However, this effect of Al_2O_3 NPs or MPs on microbial activity differed significantly and depended on the group of microorganisms. HET that made up the majority of the bacterial community in activated sludge biomass were affected to a low extent. In spite of this, the differences between the biochemical activity of HET exposed and not exposed to Al_2O_3 NPs or MPs were statistically significant (Table 1). Hence, the degrees of inhibition were calculated. They varied from 9.3% to 11.1% in the case of the exposure of HET on Al_2O_3 NPs, whereas they were from 13.3% to 22.5%, when Al_2O_3 MPs were added to the activated sludge system. These values of the degree of inhibition indicated that the mineral particles of larger size (10 µm) had a stronger impact on the biochemical activity of HET than the smaller ones (≤50 nm). What is more, the degree of inhibition of the biochemical activity of HET usually increased with the increase of the concentration of nano- or microparticles in the system (Fig. 1). It was particularly visible when Al_2O_3 MPs was added. At the same time in the case of the addition of Al_2O_3 NPs to the activated sludge system, the degrees of inhibition only slightly varied. They were actually independent of particles concentration and remained, on average, at the level of 10.2% ± 0.7%. It proves that the response of microbial activity on the chemical or material added to the system may vary and is often non-linear [22].

The biochemical activity of the autotrophic bacteria responsible for nitrogen removal from wastewater was significantly stronger affected than it was observed for the HET (compare Fig. 1 and S2) and obviously the values of biochemical activity of the nitrifying microorganisms exposed and not exposed to Al_2O_3 NPs or MPs were statistically different (Table 1). It resulted from the difference between the metabolism of autotrophic and heterotrophic microorganisms and the kinetics of their growth. Nitrifying bacteria are more sensitive to the changes of temperature, pH or toxic substances than the HET that consume biodegradable organic carbon [23,24]. However, the results presented in many studies revealed that the effect of mineral nanoparticles on nitrification was negligible at the concentration of NPs up to 10 mg L^{-1} [9,11,13,25]. For example, Wang et al. [25] determined the effect of CeO_2 NPs on the activity of enzymes responsible for nitrification, that is, ammonia monooxygenase and nitrite oxidoreductase. It occurred that the activity of these enzymes was not significantly inhibited at CeO_2 NPs concentration equal to 1 mg L^{-1}, even at the long-term exposure.

In this work, the degree of inhibition determined for the biochemical activity of nitrifying microorganisms was (with the one exception) higher than 50% that indicated on the significant effect of Al_2O_3 NPs or MPs on nitrifiers [26,27]. In the case of Al_2O_3 NPs it varied from 42.6% to 75.1%, while in the case of Al_2O_3 MPs it changed in the range from 64.1% to 84.3% dependent on the concentration of Al_2O_3 NPs or MPs (Fig. S2). From the practical point of view, the presence of Al_2O_3 NPs or MPs in the biological part of WWTPs at

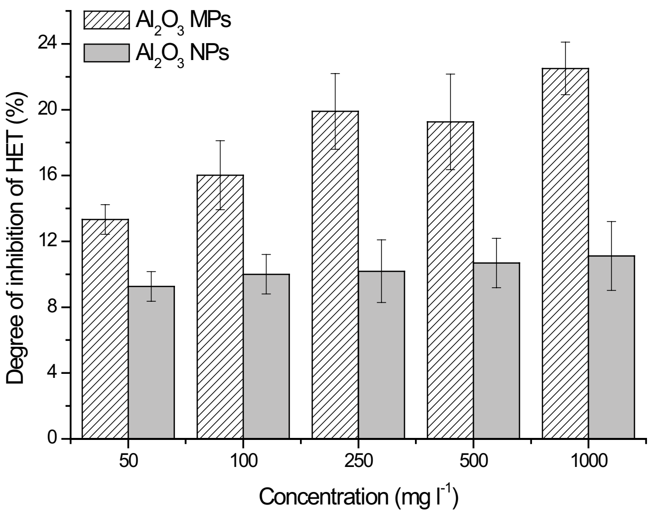

Fig. 1. Effect of Al_2O_3 MPs or NPs on the biochemical activity of heterotrophic bacteria.

Table 1
Statistical evaluation of the differences between the biochemical activity of the selected groups of activated sludge microorganisms exposed and not exposed (control tests) to Al_2O_3 MPs or NPs. The evaluation was based upon the *p*-value (one-way ANOVA)

Group of microorganisms	HET		All nitrifying microorganisms		AOB		NOB	
Type of Al_2O_3 particles	NPs	MPs	NPs	MPs	NPs	MPs	NPs	MPs
p-value	4.3×10^{-3}	2.7×10^{-3}	1.8×10^{-5}	1.0×10^{-6}	2.6×10^{-5}	1.1×10^{-7}	2.7×10^{-3}	6.7×10^{-2}

the concentrations of 50 mg L^{-1} and higher may deteriorate the removal of nitrogen from wastewater. The most probable reason for the inhibition of nitrifying bacteria by Al_2O_3 NPs or MPs was the inactivation of the enzymes catalysing the transformation of ammonium nitrogen to nitrite, and then to nitrate.

Although Chen et al. [16] found that Al_2O_3 NPs at the concentrations from 1 to 50 mg L^{-1} induced a marginal influence on the efficiency of nitrification and denitrification during the short-term exposure experiments, it cannot be excluded that any inhibition of the biochemical activity of nitrifiers might have appeared at Al_2O_3 NPs or MPs concentrations lower than 50 mg L^{-1}. It should be checked and confirmed in future studies.

The high values of the degree of inhibition determined for the nitrifiers exposed to Al_2O_3 NPs or MPs confirmed that the autotrophic microorganisms were more sensitive to the presence of chemicals (including the mineral micro- and nanomaterials) in activated sludge systems than the heterotrophic microorganisms. Similarly, as for heterotrophic bacteria, the microparticles of Al_2O_3 stronger inhibited the activity of nitrifiers than the nanoparticles of Al_2O_3 did (Fig. S2). In the case of both types of Al_2O_3 mineral particles studied the degree of inhibition increased with the increase of their concentration in the activated sludge system (Fig. S2).

Nitrification consists of two steps and for each of them, a different bacterial group is responsible. Thus, in Fig. 2 the effect of Al_2O_3 NPs or MPs on the biochemical activity of two functional groups of nitrifying microorganisms, namely AOB and NOB, is depicted. It is well seen that the addition of Al_2O_3 NPs or MPs to the activated sludge system stronger inhibited the metabolic activity of AOB compared to NOB. In the case of AOB, the degree of inhibition varied from 40.4% to 87.3%, whereas for NOB it varied from 11.8% to 69.2%. It is most probably connected with the differences in the metabolism and sensitivity of AOB and NOB to inhibitors that might have influenced the kinetics of their growth. Generally, the specific growth rate of NOB is at the same level or higher than that of AOB [23,24]. Hence, there is no accumulation of nitrite and the growth rate of AOB usually controls the overall nitrification process [23,24,28].

Analysing, the effect of the size of particles added on the degree of inhibition, it is seen that the Al_2O_3 MPs influenced on the activity of AOB to a higher extent than it was in the case of Al_2O_3 NPs (Fig. 2a). It is in agreement with the previous observations concerning the inhibition of the biochemical activity of HET (Fig. 1) as well as the inhibition of the biochemical activity of nitrifying microorganisms regarded together, that is, AOB and NOB (Fig. S2). Taking into account that the chemical composition of both materials was the same (aluminum oxide), the most probable reason of this phenomenon was that the microparticles of Al_2O_3 stronger inactivated the enzymes catalysing the transformation of carbon and nitrogen compounds in wastewater than it usually happened in the case of nanoparticles of Al_2O_3. It indicated that the application of mineral particles, particularly microparticles, to improve settling properties of activated sludge that was suggested in other works [7], should be carefully studied before their use on the industrial scale.

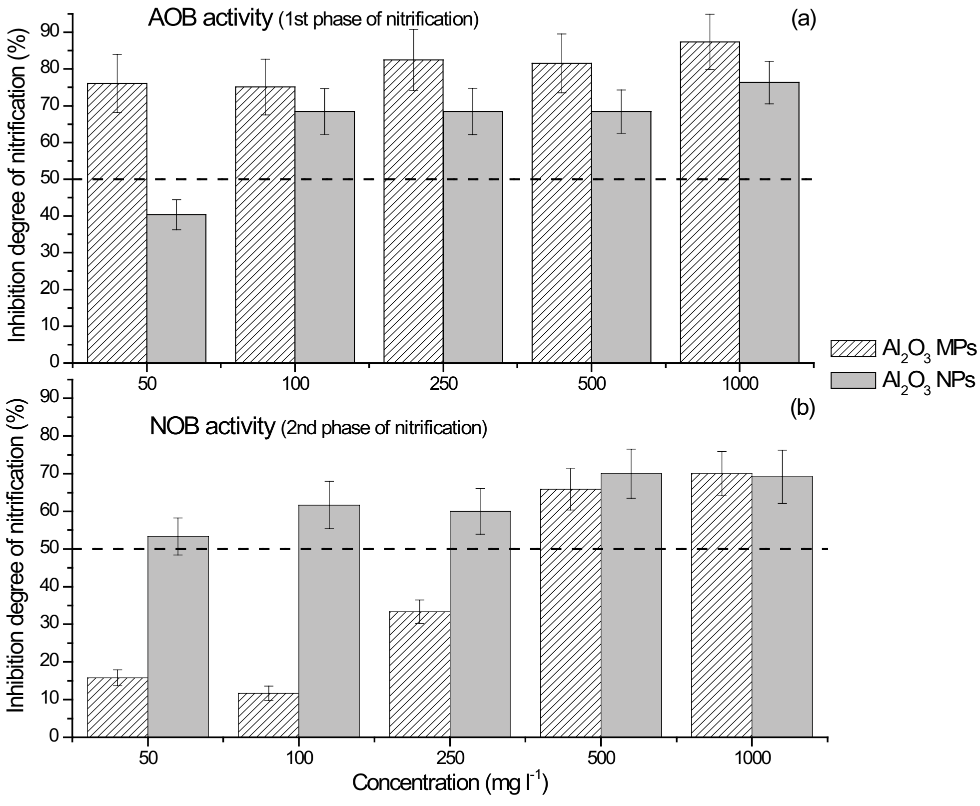

Fig. 2. Effect of Al_2O_3 MPs or NPs on the biochemical activity of nitrifying microorganisms: (a) AOB and (b) NOB.

Table 2
Mean values and standard deviations of Monod equation parameters ($\mu_{max,HET}$ and K_S) and yield coefficients (Y_{HET}) for ordinary heterotrophic organisms of activated sludge exposed and not exposed (control) to Al_2O_3 NPs or MPs

Tested compound	Mean $\mu_{max,HET}$ (h^{-1})	R^2 for $\mu_{max,HET}$ determination (–)	Mean K_S (mg O_2 L^{-1})	Mean Y_{HET} (mg VSS mg COD^{-1})
Al_2O_3 NPs	0.856 ± 0.085	0.898	14.2 ± 3.3	0.122 ± 0.024
Al_2O_3 MPs	0.865 ± 0.069	0.980	14.9 ± 3.2	0.152 ± 0.010
None (control test)	0.727 ± 0.041	0.956	15.5 ± 4.6	0.295 ± 0.052

However, the differences between the degrees of inhibition of AOB activity determined in the presence of Al_2O_3 MPs in comparison to their activity in the presence of Al_2O_3 NPs were usually lower than it was found for the HET (compare Fig. 1 and Fig. 2a). At the same time in the case of NOB, the smaller particles, that is, Al_2O_3 NPs at their lower concentrations from 50 to 250 mg L^{-1}, inhibited stronger the metabolic activity of this group of microorganisms than Al_2O_3 MPs did (Fig. 2b). At the higher concentrations of Al_2O_3 NPs or MPs (500 and 1,000 mg L^{-1}) the differences between the degrees of inhibition of NOB activity exposed either to Al_2O_3 NPs or to Al_2O_3 MPs were not statistically significant ($p = 0.522 \geq 0.05$). It indicates that in the case of the biochemical activity of NOB exposed to Al_2O_3 NPs or MPs it is difficult to unequivocally state, what type of Al_2O_3 particles, that is, NPs or MPs, influenced stronger on their activity.

3.2. Growth kinetics of HET

Growth kinetics of HET in the activated sludge systems is usually described with the use of Monod equation [23]. Thus, in this work, the values of the parameters of Monod kinetics, the maximum specific growth rate of HET ($\mu_{max,HET}$) and the half-saturation constant (K_S), were determined. Additionally, the values of the yield coefficients for the biomass of HET (Y_{HET}) were estimated. This parameter (Y_{HET}) is necessary for the mathematical description of the growth of microorganisms and belongs to one of the most sensitive parameters of activated sludge models [29,30]. The values of all determined parameters describing the growth kinetics of HET are presented in Table 2.

The presence of Al_2O_3 NPs or MPs in the activated sludge system at the concentration of 250 mg L^{-1} did not contribute to the decrease of the growth rate of HET. The values of $\mu_{max,HET}$ were higher in the tests with Al_2O_3 NPs or MPs than in the control tests (Table 2). Also, the values of K_S indicated that the addition of the nano- or microparticles of Al_2O_3 did not influence on the affinity of the HET to the organic substrate. The values of K_S in the presence of Al_2O_3 NPs or MPs were slightly lower (14.9 and 14.2 mg O_2 L^{-1}) in comparison to ones obtained in the tests without the addition of these particles, in which the mean K_S was equal to 15.5 mg O_2 L^{-1}. It is consistent with the results of the biochemical activity of HET that were inhibited by Al_2O_3 NPs or MPs to a low extent. This inhibition did not exceed 11.1% in the case of Al_2O_3 NPs and 22.5% in the case of Al_2O_3 MPs.

At the same time, the presence of Al_2O_3 NPs or MPs in the activated sludge system influenced the values of Y_{HET} and contributed to their decrease. The values of Y_{HET} were on average twice or more lower in the tests with Al_2O_3 NPs or MPs than it was found in the control tests (Table 2). It indicated that the addition of Al_2O_3 NPs or MPs affected the anabolic processes and resulted in the reduction of the synthesis of the biomass of HET. The effect of Al_2O_3 NPs or MPs on the synthesis of activated sludge biomass should be deeper analysed in the future because there is a possibility to use the mineral NPs or MPs for the reduction of the excess sludge released from the WWTPs. Moreover, all the values of determined parameters, particularly the stoichiometric coefficients, might be used in modelling and simulation of biological wastewater treatment processes, in which Al_2O_3 NPs or MPs are added to the activated sludge system.

4. Conclusions

The addition of Al_2O_3 NPs or MPs to the activated sludge system influences on the biochemical activity and growth kinetics of the functional group of microorganisms as it was summarized below.

- In the activated sludge system the biochemical activity of HET is inhibited to a low extent even at high concentrations of Al_2O_3 NPs or MPs up to 1,000 mg L^{-1}. The degree of inhibition does not usually exceed 20%. Aluminium oxide NPs or MPs do not decrease the specific growth rate of HET either.
- The biochemical activity of the nitrifying microorganisms significantly decreases in the presence of Al_2O_3 NPs or MPs in the activated sludge system. The degree of inhibition varied from 42.6% to 84.3% and usually increased with the increase of the concentration of Al_2O_3 NPs or MPs in the activated sludge system. Out of all the nitrifying microorganisms, the group of AOB is affected stronger by Al_2O_3 NPs or MPs than the group of NOB.
- The particles of smaller size, that is, Al_2O_3 NPs, inhibit the biochemical activity of HET and nitrifying microorganisms, particularly AOB, to a higher extent than Al_2O_3 MPs do.
- The presence of Al_2O_3 NPs or MPs contributes to the reduction of the synthesis of activated sludge biomass. It indicates on the potential use of these materials to decrease the amount of the excess sludge in the activated sludge systems. Due to the inhibition of nitrifying organisms by Al_2O_3 NPs or MPs, the concentration of these micro- or nanoparticles should be carefully selected before their application in the full-scale WWTPs.

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References

[1] X. Huangfu, Y. Xu, C. Liu, Q. He, J. Ma, Ch. Ma, R. Huang, A review on the interactions between engineered nanoparticles with extracellular and intracellular polymeric substances from wastewater treatment aggregates, Chemosphere, 219 (2019) 766–783.
[2] N. Javid, M. Malakootian, Removal of bisphenol A from aqueous solutions by modified-carbonized date pits by ZnO nano-particles, Desal. Water Treat., 95 (2017) 144–151.
[3] Ch. Santhosh, V. Velmurugan, G. Jacob, S. Kwan Jeong, A.N. Grace, A. Bhatnagar, Role of nanomaterials in water treatment applications: a review, Chem. Eng. J., 306 (2016) 1116–1137.
[4] X. Qu, P.J.J. Alvarez, Q. Li, Application of nanontechnology in water and wastewater treatment, Water Res., 37 (2013) 3931–3946.
[5] G. You, P. Wang, J. Hou, Ch. Wang, Y. Xu, L. Miao, B. Lv, Y. Yang, Z. Liu, F. Zhang, Insights into the short-term effects of CeO_2 nanoparticles on sludge dewatering and related mechanism, Water Res., 118 (2017) 93–103.
[6] X. Xiao, S. Liu, X. Zhang, S. Zheng, Phosphorus removal and recovery from secondary effluent in sewage treatment plant by magnetite mineral microparticles, Powder Technol., 306 (2017) 68–73.
[7] A. Masłoń, J.A. Tomaszek, J. Zamorska, M. Zdeb, A. Piech, I. Opaliński, Ł. Jurczyk, The impact of powdered keramsite on activated sludge and wastewater treatment in a sequencing batch reactor, J. Environ. Manage., 237 (2019) 305–312.
[8] J. Chen, Y.Q. Tang, Y. Li, Y. Nie, L. Hou, X.Q. Li, X.L. Wu, Impacts of different nanoparticles on functional bacterial community in activated sludge, Chemosphere, 104 (2014) 141–148.
[9] Y. Wang, P. Westerhoff, K.D. Hristovski, Fate and biological effects of silver, titanium dioxide, and C_{60} (fullerene) nanomaterials during simulated wastewater treatment processes, J. Hazard. Mater., 201–202 (2012) 16–22.
[10] S. Wang, M. Gao, Z. She, D. Zheng, Ch. Jin, L. Guo, Y. Zhao, Z. Li, X. Wang, Long-term effects of ZnO nanoparticles on nitrogen and phosphorus removal, microbial activity and microbial community of a sequencing batch reactor, Bioresour. Technol., 216 (2016) 428–436.
[11] G. Qiu, K. Wirianto, Y. Sun, Y.P. Ting, Effect of silver nanoparticles on system performance and microbial community dynamics in a sequencing batch reactor, J. Cleaner Prod., 130 (2016) 137–142.
[12] S. Simelane, J.C. Ngila, L.N. Dlamini, The fate, behavior and effect of WO_3 nanoparticles on the functionality of an aerobic treatment unit, Environ. Nanotechnol. Monit. Manage., 8 (2017) 199–208.
[13] H. Zhou, G. Xu, Effect of silver nanoparticles on an integrated fixed-film activated sludge–sequencing batch reactor: performance and community structure, J. Environ. Sci., 80 (2019) 229–239.
[14] N.Q. Puay, G. Qiu, Y.P. Ting, Effect of Zinc oxide nanoparticles on biological wastewater treatment in a sequencing batch reactor, J. Cleaner Prod., 88 (2015) 139–145.
[15] P. Cervantes-Avilés, G. Cuevas-Rodríguez, Changes in nutrient removal and flocs characteristics generated by presence of ZnO nanoparticles in activated sludge process, Chemosphere, 182 (2017) 672–680.
[16] Y. Chen, Y. Su, X. Zheng, H. Chen, H. Yang, Alumina nanoparticles-induced effects on wastewater nitrogen and phosphorus removal after short-term and long-term exposure, Water Res., 46 (2012) 4379–4386.
[17] A. Lazareva, A.A. Keller, Estimating potential life cycle releases of engineered nanomaterials from wastewater treatment plants, ACS Sustainable Chem. Eng., 2 (2014) 1656–1665.
[18] J. Surmacz–Górska, K. Gernaey, C. Demuynck, P. Vanrolleghem, W. Verstraete, Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements, Water Res., 30 (1996) 1228–1236.
[19] E. Liwarska-Bizukojc, D. Gendaszewska, Removal of imidazolium ionic liquids by microbial associations: study of the biodegradability and kinetics, J. Biosci. Bioeng., 115 (2013) 71–75.
[20] J. Kappeler, W. Gujer, Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modeling, Water Sci. Technol., 25 (1992) 125–139.
[21] APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, 22nd ed., American Public Health Association-American Works Water Association-Water Environment Federation, Washington DC, 2012.
[22] G.M. Rand, P.G. Wells, L.S. McCarty, Introduction to Aquatic Toxicology, G.M. Rand, Ed., Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment, 2nd ed., Taylor & Francis, New York, 1995, pp. 3–67.
[23] W.W. Eckenfelder, J.L. Musterman, Activated Sludge Treatment of Industrial Wastewater, Technomic Publishing Company Inc., Lancaster PA, 1995.
[24] C.P. Leslie Grady Jr., Glen T. Daigger, Nancy G. Love, Carlos D.M. Filipe, Biological Wastewater Treatment, 3rd ed., CRC Press, Boca Raton, 2011.
[25] X. Wang, M. Zhu, N. Li, S. Du, J. Yang, Y. Li, Effects of CeO_2 nanoparticles on bacterial community and molecular ecological network in activated sludge system, Environ. Pollut., 238 (2018) 516–523.
[26] OECD Chemical Group, Activated Sludge, Respiration Inhibition Test, Method 209, OECD Revised Guidelines for Tests for Ready Biodegradability, OECD, Paris, 1984.
[27] D.J. Hoffman, B.A. Rattner, G.A. Burton Jr., J. Cairns Jr., Handbook of Ecotoxicology, CRC Press LLC, Boca Raton, 2002.
[28] M. Henze, P. Harremoës, J. Jansen, E. Arvin, Wastewater Treatment. Biological and Chemical Processes, Springer, Berlin, Heidelberg, 2002.
[29] E. Liwarska-Bizukojć, R. Biernacki, Identification of the most sensitive parameters in the activated sludge model implemented in BioWin software, Bioresour. Technol., 101 (2010) 7278–7285.
[30] B. Petersen, K. Gernaey, M. Henze, P.A. Vanrolleghem, Calibration of Activated Sludge Models: A Critical Review of Experimental Designs, S.N. Agathos, W. Reineke, Eds., Biotechnology for the Environment: Wastewater Treatment and Modeling, Waste Gas Handling, Kluwer Academic Publishers, Dordrecht, 2003, pp. 101–186.

Supplementary information

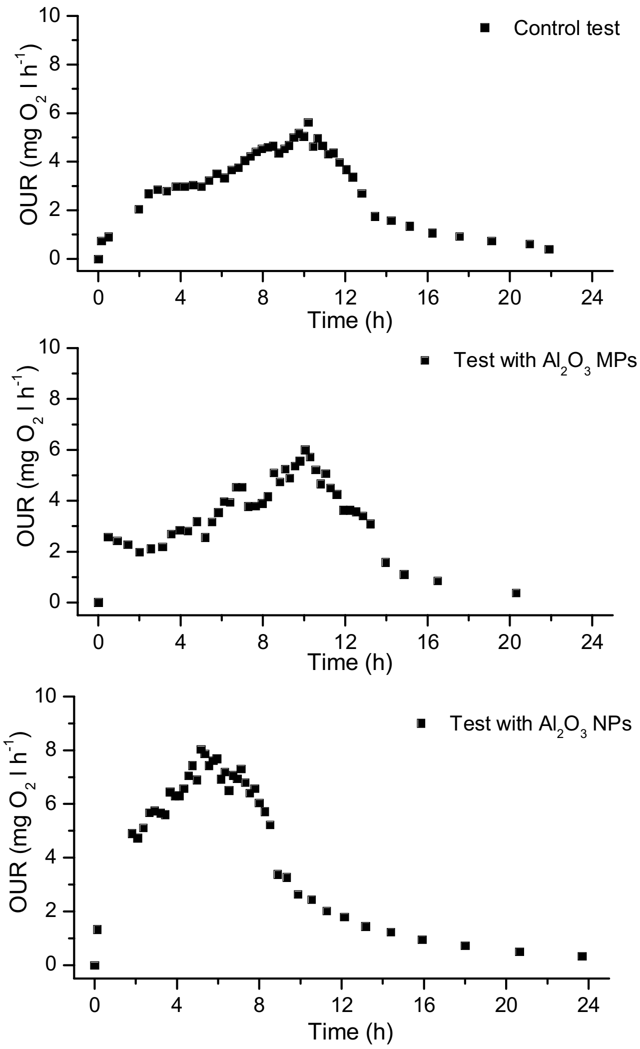

Fig. S1. Example of the changes of OUR in time obtained in the second type of OUR tests.

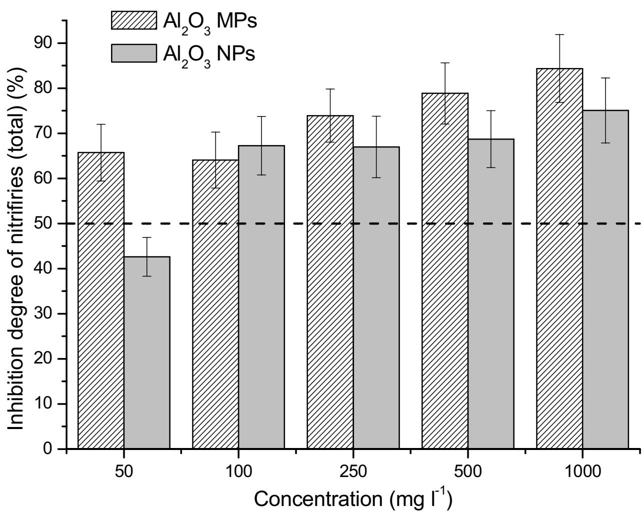

Fig. S2. Effect of Al_2O_3 NPs or MPs on the biochemical activity of nitrifying microorganisms.