Archives of Environmental Protection
Vol. 46 no. 4 pp. 54–58

PL ISSN 2083-4772
DOI 10.24425/aep.2020.135764

Manganese dioxide (MnO_2) nanoparticles influence on the nitrification and anammox activity

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Keywords: wastewater, nanoparticles, nitrification, anammox, manganese dioxide.

Abstract: The anammox (anaerobic ammonia oxidation) process is one of the most efficient processes of nitrogen removal from wastewater. Although there are some applications of anammox-based technologies, it is still difficult to apply this process widely because of the high optimal temperature around 30–40°C. Thus, the main objective of this study was to evaluate the short-term effects of MnO_2 on the anammox and nitrification process activity at a wide range of temperatures between 10 and 30°C, using statistical methods based on the central composite design (CCD). The influence of MnO_2 on anammox and nitrification activity, suspended biomass from the laboratory-scale sequencing batch reactor (SBR), and activated sludge from WWTP, respectively, was used. MnO_2 concentration range was set between 15 and 85 mg/L, and the temperature range was set between 10 and 30°C. Anammox and nitrification process activity was measured based on the batch test and oxygen uptake rate (OUR), respectively. The results were statistically analyzed. Results revealed that nanoparticles can slightly improve anammox activity by several percent, by up to 10%, but in most cases MnO_2 influence was insignificant. The optimal concentration for the anammox stimulation at temperatures below 20°C was evaluated between 40 and 60 mg/L, corresponding to 36 and 56 mg/g VSS. Manganese oxides contribution in the nitrogen removal processes was proved and they should be considered in the field of the anammox process. Thus, further studies are suggested to investigate the long-term effects of MnO_2 on the low-temperature anammox process, overcoming possibility of inhibition.

Introduction

Partial nitrification – anammox (anaerobic ammonia oxidation) process was recognized as an efficient and beneficial alternative for the conventional nitrification-denitrification technology to treat high strength ammonia wastewater. The implementation of this technology in the mainstream of the municipal wastewater treatment plant (WWTP) will offer an opportunity to achieve energy-neutral or energy positive wastewater treatment (Cao et al., 2017). Although there are some applications of anammox-based technologies in practical wastewater treatment, it is still difficult to apply this process widely because of high optimal temperature (30–40°C). An effective low-temperature anammox process seems to be one of the most challenging but profitable processes in wastewater treatment. Another important challenge is the suppression of nitrite oxidizing bacteria (NOB) and the promotion of ammonia oxidizing bacteria (AOB) and anammox bacteria at low nitrogen influent concentrations.

Therefore, finding a way to support anammox bacteria to perform an effective nitrogen removal process at low temperature (< 20°C) has been the focus of recent investigations. Several interesting methods to improve anammox efficiency have been described in the last years. One of them is manganese oxides addition (Li et al., 2018; Xu et al., 2018), including its micro- (Qia et al., 2012), and nanopowder form (Xu et al., 2019). Nanoparticles possess their own unique physical and chemical properties due to their nanoscale size and high surface area. Nanomaterials are able to change biomolecules conformation state and thus their catalytic properties (Pavlidis et al., 2014).

Manganese dioxide (MnO_2) was studied for its catalytic activity in chemical reactions, but it is also used in biological processes. Manganese is an essential element of the anammox metabolism and significantly affects the synthesis of heme c, engaged in the energy transduction processes. Mn-oxides' abilities to enhance nitrogen removal by anammox process were previously demonstrated in the natural ecosystems. Thamdrup and Dalsgaard (2002) deduced, based on the anammox reaction rate in the sediment samples, that anammox can be promoted over denitrification as a sink of nitrate in sediments rich in manganese oxides. According to Engström et al. (2004), manganese oxides enhanced buffering capacity and acted as an oxidant, significant in marine sediments with the limited organic carbon. It is suggested that the availability of manganese may stimulate and support N_2 formation and nitrogen removal by anammox process compared to denitrification in surface marine sediments (Engström et al., 2004; Hulth et al., 1999). Kartal et al. (2007) proved that

anammox bacteria – Candidatus *Kuenenia stuttgartiensis* has a versatile lifestyle, which allows it to employ as an electron acceptor ferrous and manganese oxides. The effects of micro-scale MnO_2 addition (50 g/L) on nitrogen conversion and anammox process in a technological system was explored by Qiao et al. (2012). The nitrogen removal rate of the reactor without MnO_2 addition reached only 464.6 g N/m^3·d (with nitrogen loading rate of 634.9 g N/m^3·d), while the nitrogen removal rate of the reactor with MnO_2 addition increased to 920.9 g N/m^3·d (with nitrogen loading rate of 1059.6 g N/m^3·d). The average crude enzyme activity of the reactor with MnO_2 addition was 78.2% higher (2012). Other study revealed that nitrogen removal rate during long-term cultivation can be enhanced from 12.0 g N/m^3·d to 13.1 g N/m^3·d by 10 mg/L of conventional MnO_2 (2018) and from 12.2 g N/m^3·d to 12.9 g N/m^3·d by 200 mg/L of nano-scale MnO_2 (2019).

However, these studies (Qiao et al., 2012; Xu et al., 2018; 2019) were conducted at high temperature (35°C), while at the mainstream of the WWTP, the anammox process is desired to work at much lower temperatures (< 20°C). Moreover, in case of the single-stage partial nitrification-anammox process, the operational strategy is usually focused not only on the anammox process but also on the promotion of AOB over NOB (Kouba et al., 2017). At the same time, anammox bacteria cannot be cultivated as a pure culture and they coexist with other nitrogen cycle microorganisms (Ziembińska-Buczyńska et al., 2019) and the role of these bacteria was omitted in previous works.

Thus, the main objective of the presented study was to evaluate the short-term effects of the MnO_2 on the anammox process activity at a wide range of temperatures (10–30°C), using statistical methods based on the central composite design (CCD). Additionally, the nitrification activity under MnO_2 exposure was analyzed.

Materials and methods

MnO_2 nanoparticles characteristics

Commercially available MnO_2 nanopowder (US Research Nanomaterials, Inc., USA) used in all assays was characterized by: particles size < 50 nm, specific surface area: 55 m^2/g, elemental analysis (percentage by weight): Mn > 59%, Cu < 0.028%, Pb < 0.019%, Ca < 0.085%, Fe < 0.014%, Mg < 0.068%, K < 0.039%, Na < 0.068%.

Anammox activity determination

Suspended anammox biomass from the laboratory-scale sequencing batch reactor (SBR) was used in these experiments. The reactor was operated at a temperature of 31 ± 1°C, pH 7.6 ± 0.2, and was fed with a mineral medium with a total nitrogen loading rate of 0.784 ± 0.048 g N/L·d. Mineral medium composition was adapted from van de Graaf et al. (1996): 1.2 g NH_4Cl/L, 2.1 g $NaNO_2$/L, 0.048 g $KHCO_3$/L, 0.041 g KH_2PO_4/L, 0.228 g $MgSO_4 \cdot 7\ H_2O$/L, 0.007 g $FeSO_4 \cdot 7\ H_2O$/L, 0.004 g EDTA/L.

Based on preliminary studies, the MnO_2 concentration range was set between 15 and 85 mg/L, and the temperature range was set between 10 and 30°C. All tests were triplicated. Tests were performed according to the methodology described in previous work (Tomaszewski et al., 2019) in batch reactors, with a working volume of 100 mL, with initial substrate concentrations equal to 30 mg NH_4-N/L and 30 mg NO_2-N/L, and volatile suspended solids (VSS) concentration of 1.1 ± 0.3 g VSS/L. The samples from the batch test reactors were periodically collected for the NH_4-N and NO_2-N concentrations measurement at period adapted to the reaction time, determined by the temperature used in the experiment (2.5–30 h). Specific anammox activity (g N/g VSS·d) was calculated based on the decrease of the nitrogen in the linear range of substrates removal and MnO_2 influence was evaluated as a percentage of SAA in relation to the control.

The experiments were planned according to central composite design (CCD) to obtain a mathematical relationship for the independent and simultaneous influence of temperature and the MnO_2 concentration on the anammox activity. Statistical software (STATISTICA StatSoft®) was used for the analysis of the CCD experiment. Based on the regression analysis, the coefficients of the second-order polynomial equation were calculated. The mathematical relationship of the influence of independent and interactive temperature and MnO_2 concentration on the activity was approximated by the polynomial quadratic formula. Finally, the obtained mathematical model was tested using the analysis of variance (ANOVA).

Nitrification activity determination

Conventional activated sludge originated from a municipal wastewater treatment plant located in Southern Poland. Nitrification activity was measured based on the oxygen uptake rate (OUR) measurements, according to the methodology described by Surmacz-Górska et al. (1996). A phosphate buffer was added to the biomass (to reach a final concentration of 0.14 g KH_2PO_4/L and 0.75 g K_2HPO_4/L) and the pH was adjusted at 7.5 using 10% HCl or 10% NaOH. The samples were placed in vessels (volume of 120 mL), with an oxygen sensor (N5221 Elwro) connected with oxygen level recorder (Line Recorder T2 4620). The tests were performed in triplicate, at two temperatures: 10 and 20°C, with three nanoparticles concentrations: 100, 200 and 300 mg/L. An average total suspended solids (TSS) concentration was 4.5 ± 0.9 g TSS/L. The obtained results allowed to calculate the respiration activity in mg O_2/L·h.

Results and discussion

Anammox

The influence of MnO_2 was studied for temperatures ranging from 10 to 30°C, which are lower than the optimum temperature for the anammox bacteria. The MnO_2 effects were evaluated as a relative activity (%) in relation to the control experiments without MnO_2 addition. A second-order polynomial equation (equation 1) was successfully determined to describe the temperature and MnO_2 concentration influence on the anammox process, with the coefficient of determination (R2) equal to 0.91. The analysis of variance test was used to evaluate the obtained model (Table 1). The "Lack of fit" F-value of 2.463 and p-value of 0.239 implying that model is suitable to describe the relationship between tested parameters. The results of the ANOVA test confirmed also that MnO_2 nanoparticles affect anammox biomass activity.

$$\text{activity (\%)} = 108.67 - 2.64 \cdot T + 0.9 \cdot MnO_2 + \\ + 0.1 \cdot T^2 - 0.02 \cdot MnO_2^2 - 0.03 \cdot T \cdot MnO_2 \quad (1)$$

Anammox activity calculated based on the regression model and empirical results is summarized in Figure 1. Predictions of the regression model obtained from the CCD are consistent with the results obtained in the batch experiments. In most cases, MnO_2 influence on the anammox activity was insignificant and varied between 96 and 106% of relative activity. However, the maximal inhibition of the anammox activity by about 17 ± 2% was demonstrated after the addition of 85 mg MnO_2/L (77 mg/g VSS) at 20°C, while a maximal enhancement by about 10 ± 3% was caused by 50 mg MnO_2/L (45 mg/g VSS) at 30°C. The same concentration (50 mg/L) lead to 9% stimulation at 10˚C. This stimulation values are similar to the previous study conducted by Xu et al. (Xu et al., 2018; 2019). They revealed that nitrogen removal rate during long-term cultivation at 35°C can be enhanced by 9% with 10 mg/L of MnO_2 (Xu et al., 2018) and by 6% with 200 mg/L of nano-MnO_2 (Xu et al., 2019). Taking into account biomass concentration (18.3 and 19.2 g VSS/L, respectively) the MnO_2 doses were ca. 0.5 and 10 mg/g VSS, much lower than in our short-term study. Li et al. (2018) and Qiao et al. (2012) observed that Mn can penetrate into anammox bacteria cells, indicating that Mn might be accumulated during the long operation, which explains the difference between short- and long-term effects. However, a significant difference between non-nano-scale and nano-scale MnO_2 concentration observed by Xu et al. (2018; 2019) has not been explained, but proved that particle size does matter. The nitrogen removal rate stimulation was connected with the growth in the extracellular polymeric substances (EPS) concentration, heme c content and relative abundance of anammox bacteria, but these experiments (Xu et al., 2018; 2019) were conducted in a single reactor with MnO_2 concentration growing in time for 175 days. For this reason, the observed changes and nitrogen removal improvement can be also associated with biomass adaptation and development in the new environment (reactor).

Figure 2 presents a contour plot of the response surface obtained based on a mathematical model. It clearly demonstrates that MnO_2 effects depend on its concentration and environment temperature. Higher concentrations of the nanoparticles are required to achieve stimulation effect on the anammox activity at lower temperature (< 20°C), while too high concentrations (> 70 mg/L) at temperatures above 20°C have the strongest inhibition effect. At the same time, we can observe the inflection line on the response surface between ca. 16 and 22°C. This is consistent with previous studies which revealed that temperature near 15–20°C constitutes some kind of breaking point for the metabolism of anammox bacteria (Lotti et al., 2015; Tomaszewski et al., 2017).

Several types of research tried to reveal Mn-oxide's contribution in the anammox process. Qiao et al. (2012) demonstrated that Mn is favorable for enzyme activity inside the cells and that it can substitute iron and its function. Fe and Mn ions are essential elements of anammox metabolism and significantly affect the synthesis of heme c, which participates in energy metabolism and anammox bacteria proliferation. On the other hand, one of the first studies showed that metal ions could be employed as an electron acceptor in the

Table 1. ANOVA for quadratic model (F – Fisher's F value; p – probability value)

Source	F	p
MnO_2 linear	39.305	0.008
MnO_2 square	12.263	0.039
Temperature linear	0.456	0.548
Temperature square	27.898	0.013
Interaction	17.647	0.024
Lack of fit	2.463	0.239

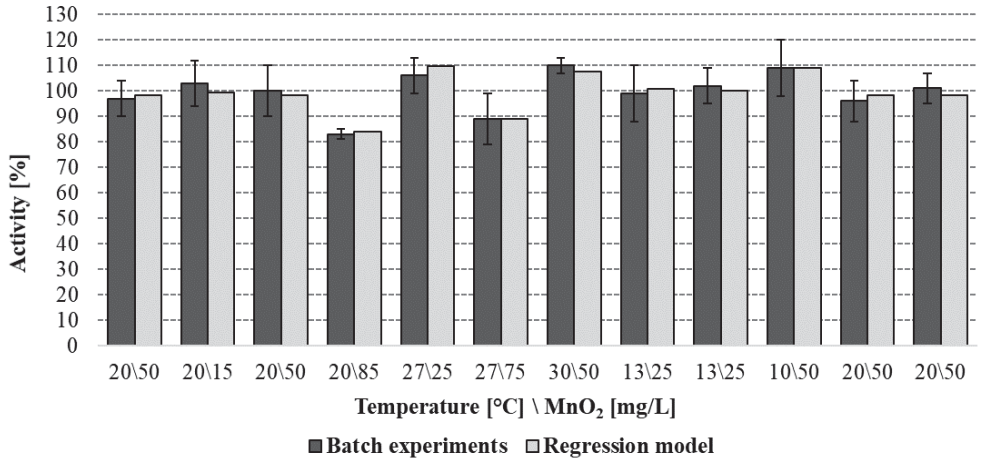

Fig. 1. Influence on the specific anammox activity (% in relation to the control without the MnO_2 addition) obtained in the batch experiments and calculated based on the regression model

metabolism of anammox bacteria (Kartal et al., 2007, Strous et al., 2006). The results of the above studies demonstrated that Mn-oxides favored N_2 production and anammox process in the natural ecosystems. This thesis was currently supported by Chen et al. (2019), who detected simultaneous oxidation of NH_4^+ and MnO_2 reduction, coupled with NO_2^-, NO_3^- and N_2 production. Besides feammox, Mn – mediated anaerobic ammonium oxidation (termed Mn-anammox) is considered as an alternate and insufficiently known microbial pathway in the nitrogen cycle (Luther et al., 1997; Chen et al., 2019).

Nitrification

The respiratory activity of the nitrifying bacteria at two temperatures, with different concentrations of the nanoscale MnO_2, is shown in Figure 3. Taking into consideration a low influence on the anammox activity in most experiments conducted for concentrations from 15 to 85 mg/L, the values above this range were chosen (100, 200, 300 mg/L). The effect of the MnO_2 was evaluated as a percentage of activity in relation to the control without the addition of the nanoparticles.

The results obtained at 20°C revealed no statistically significant influence on the respiration activity of nitrifying bacteria. A relative activity varies between 91 and 104%. Statistically significant inhibition (by 25%) was noted only at 10°C with the highest tested concentration – 300 mg/L. The activated sludge used in this study, was adapted to temperature ca. 15°C. Therefore, it can be suspected that it was less resistant at a temperature below this value. There is evidence in the literature of Mn-oxide's contribution to the nitrogen transformation (Luther et al., 1997), but there is lack of reports about its influence on nitrifying bacteria in the aquatic environment. Probably, these concentrations in hundreds of mg/L have not been studied, because they are unrealistically high, both in the environment and in WWTP. From the presented research point of view, it can be considered that concentrations capable of supporting the anammox process do not affect nitrifying bacteria activity under short-term exposure. However, because the partial nitrification – anammox process requires the promotion of ammonia oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB), further studies are needed to investigate the effects of on the AOB and NOB separately.

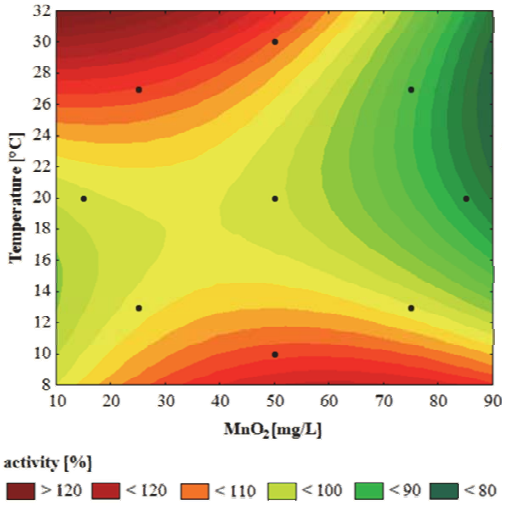

Fig. 2. Contour plot of the simultaneous effect of temperature and MnO_2 on the anammox activity (% in relation to the control without the MnO_2 addition)

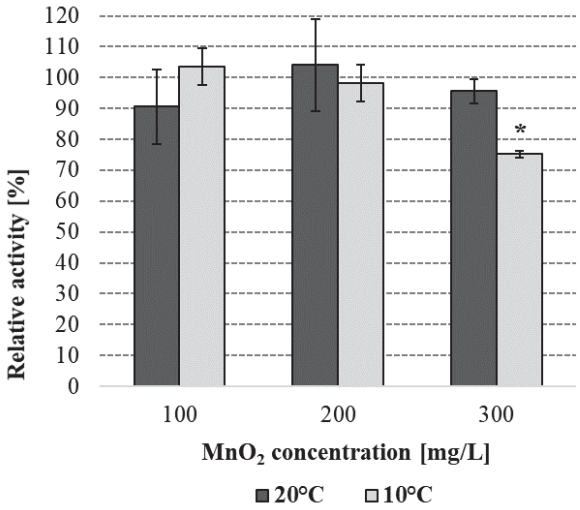

Fig. 3. Relative respiratory activity of the nitrifying bacteria at different temperatures under exposure on MnO_2 nanoparticles. Bars represent standard error; * means statistically significant difference with control at $p < 0.05$

Conclusions

Short-term effects of nano-scale MnO_2 on the nitrification and anammox processes activity were studied. Nanoparticles can slightly improve anammox activity by several percent (up to 10%), but in most cases the MnO_2 influence was insignificant. The optimal concentration for the anammox stimulation at low temperatures (< 20°C) was evaluated between 40 and 60 mg/L (36–55 mg/g VSS). On the other hand, too high concentrations had an inhibitory effect on the anammox (85 mg/L; 77 mg/g VSS) and nitrification (300 mg/L) activity. These findings cannot be assumed as satisfying for supporting the low-temperature anammox process. However, manganese and manganese oxides contribution in the nitrogen removal processes was proved and they should be still considered in this field, including low--temperature anammox process. Thus, further studies are required to investigate the long-term effects of MnO_2 on the anammox activity at low temperatures in the continuous experiment.

Acknowledgments

The study was financed by the National Science Centre, Poland: UMO-2017/25/N/NZ9/01159 and supported by the Foundation for Polish Science (FNP).

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Wpływ nanocząstek tlenku manganu (MnO_2) na aktywność procesów nitryfikacji i anammox

Streszczenie: Proces anammox (beztlenowe utlenianie amoniaku) jest procesem efektywnego usuwania azotu ze ścieków. Pomimo, że istnieje wiele technologi wykorzystujących proces anammox, jego zastosowanie nadal jest ograniczone ze względu na wysoką optymalną temperaturę (około 30–40°C). W związku z tym, celem tej pracy była ocena krótkoterminowego wpływu MnO_2 na aktywność procesów anammox i nitryfikacji w zakresie temperatur od 10 do 30°C, przy użyciu metod statystycznych. Do badań wykorzystano biomasę anammox pobraną z laboratoryjnego sekwencyjnego reaktora porcjowego oraz biomasę bakterii nitryfikacyjnych pochodzącą z komunalnej oczyszczalni ścieków. Badania prowadzono przy zastosowaniu stężeń MnO_2 z zakresu od 15 do 85 mg/l oraz temperatur pomiędzy 10–30°C. Aktywność procesu anammox zbadano przy pomocy testów porcjowych, natomiast do zbadania aktywność procesu nitryfikacji wykorzystano pomiar szybkości zużycia tlenu. Wyniki wykazały, że nanocząstki MnO_2 mogą poprawić aktywność procesu anammox o kilka procent (nawet o 10%). Optymalne stężenie MnO_2 dla stymulacji procesu anammox w temperaturach poniżej 20°C wynosiło między 40 a 60 mg/l, co odpowiada 36 i 56 mg/g s.m.o. Niniejsze badania udowadniają, że dodatek MnO_2 może powodować wzrost aktywności procesu anammox przy jednoczesnym obniżeniu temperatury. Dlatego sugeruje są dalsze badania w celu zbadania długoterminowego wpływu nanocząstek MnO_2 na niskotemperaturowy proces anammox.