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Short-term effects of reduced graphene oxide on the anammox biomass activity at low temperatures

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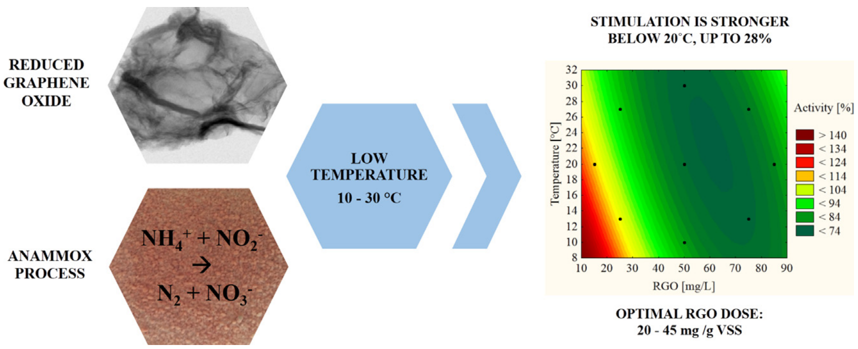
HIGHLIGHTS

- The maximum 28% activity stimulation was obtained at 13 °C and 15 mg RGO/L
- The effect depends on the RGO dose per biomass unit, not on its concentration
- The optimal dose was evaluated as 20 to 45 mg RGO/g VSS
- The stimulation effect is stronger at temperatures below 20 °C

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic ammonium oxidation (anammox) is an efficient process for nitrogen removal from wastewater, but its common use is limited by its relatively high optimal temperature (30 °C). One of the major bottlenecks of the implementation of mainstream PN/A process is the low activity of the anammox bacteria at low temperature. Due to this reason over the past years, numerous researchers have attempted to overcome this limitation. Recently it was shown that the reduced graphene oxide (RGO) can accelerate the anammox bacteria activity. However all these studies were performed at high temperatures (over 30 °C). Thus, in this study, supporting the anammox process at low temperatures (10–30 °C) by the RGO was investigated for the first time. The statistical analysis confirmed that RGO significantly affects the anammox activity. The stimulation effect of RGO on the anammox bacteria activity is of particular importance at low temperatures, when drastic decrease in process activity is observed at temperatures below 15 °C. The short-term experimental results demonstrated stimulation of the anammox activity at 13 °C, up to 28% by 15 mg RGO/L, but concentrations above 40 mg RGO/L caused the process inhibition, up to 30% with 50 mg RGO/L. However, the effect of RGO probably depends on the nanomaterial dose per biomass unit and the optimal range of this value was evaluated as 20 to 45 mg RGO/g VSS (volatile suspended solids).

1. Introduction

The anammox (anaerobic ammonium oxidation) process is recognised to be the most energy efficient and environmentally-friendly process of nitrogen removal from high strength ammonia wastewater (Garrido et al., 2013). In the anammox process, ammonium

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is anaerobically oxidised with nitrite used as the electron acceptor by a unique bacterial phylum - *Planctomycetes*. In order to achieve an adequate nitrite-to-ammonium ratio (1.32:1), a part of ammonium must be oxidised to nitrite in the partial nitritation process. Compared to conventional processes (nitrification and denitrification), nitritation (ammonium oxidation to nitrite)/anammox (PN/A) has advantages in the reduction of the oxygen demand (60% reduction of aeration costs), the alkalinity demand for nitrogen removal is reduced by ca. 45%, it does not require any organic carbon source (reducing the exogenous carbon source required for main stream), and additionally, it allows to achieve a 80% reduction of the excess sludge (Cao et al., 2017; Tomaszewski et al., 2017a). Moreover, during this process a higher removal rate is obtained, it requires a smaller bioreactor volume, and it emits a smaller amount of greenhouse gases (Feng et al., 2017; Tomaszewski et al., 2017a). The partial nitritation/anammox processes have been already applied at full scale for treatment of reject water of anaerobic sludge digester, which allow to reduce total electrical energy consumption by 40–50% with biogas utilization (Siegrist et al., 2008). Up to now, there are more than two hundred full scale applications of anammox-based technologies (Lackner et al., 2014; Ali and Okabe, 2015; Cao et al., 2017), usually treating digestate, landfill leachate, or reject water (De Cocker et al., 2018). Generally, most of the reported systems were operated at temperatures exceeding 25 °C and the influent nitrogen concentration over 100 mg/L (Lotti et al., 2015a). Common use of this technology and its implementation in the mainstream of the municipal wastewater treatment plant (WWTP) (with lower temperatures and lower nitrogen concentrations) will offer an opportunity to achieve energy neutral/positive wastewater treatment (Siegrist et al., 2008; Lotti et al., 2015b; Cao et al., 2017). The application of PN/A in the mainstream of the WWTP is facing the challenge of a drastic decrease of the anammox bacteria growth rate and activity at temperatures below 15 °C, and additionally of seasonal temperature variations of municipal wastewater. Another big challenge is the suppression of nitrite oxidizing bacteria (NOB) and the promotion of growth of anammox bacteria at low nitrogen influent concentrations. Another thing is a high C/N ratio of municipal wastewater, which can result in a large fraction of heterotrophic bacteria in the sludge. And the final challenge will be the need to meet strict discharge standards of the effluent (Gilbert et al., 2015; Lotti et al., 2015a; Cao et al., 2017; De Cocker et al., 2018). An effective low-temperature anammox process seems to be one of the most challenging but profitable processes in wastewater treatment.

One of the major bottlenecks of the implementation of mainstream PN/A process is low activity of anammox bacteria at low temperature. Thus, the intensification of the anammox activity is the area for further studies (Cao et al., 2017). Numerous researchers have studied methods to increase the anammox efficiency, for example by ultrasounds (Yu et al., 2013), ferrous iron (Bi et al., 2014), or quinoid redox mediators (RM) (Qiao et al., 2014). RM are organic molecules that can reversibly be oxidised and reduced, that accelerates reactions by lowering the activation energy of the enzymatic reaction. However, the field of environmental sciences reflects a fast development of nanotechnology. The role of nanomaterials in water and wastewater treatment is mainly connected with their adsorptive, photocatalytic, and antimicrobial properties (Santhosh et al., 2016). Several recent studies indicate that the activity of anammox bacteria can be accelerated by a few kinds of nanoparticles. They include: divalent ferrous ion (Liu and Ni, 2015), active carbon (Laureni et al., 2015), manganese oxide (Qiao et al., 2012), and graphene materials (Wang et al., 2013; Yin et al., 2015, 2016). Graphene is an atom-thick sheet consisting of carbon atoms in a closely packed honeycomb lattice; it is called "the material of the future". Graphene and its derivatives (graphene oxide (GO) and reduced graphene oxide (RGO)) have unique structures and properties, which make them interesting materials in fields of physics, chemistry, and materials science. GO contains functional groups, such as carboxyl, hydroxyl, and epoxide, while RGO is its counterpart holding mainly carboxyl groups, at the periphery, which make them hydrophobic (Yang et al., 2013). The same recent studies demonstrated that graphene nanomaterials could enhance the anammox process efficiency. Wang et al. (2013) reported that a 100 mg/L GO addition could increase the total nitrogen removal rate of annamox by 10%, while Yin et al. (2015, 2016) found that 100 mg/L RGO led to a 17% increase of the total nitrogen removal rate and that the anammox process start-up could be reduced from 67 to 49 days by its addition. The increase of process activity is associated with the enhancement of key anammox enzymes - the hydrazine dehydrogenase (HDH), nitrite reductase (NIR), and nitrate reductase (NAR) (Yin et al., 2015, 2016). However, a too high RGO concentration (150 mg/L) inhibited the anammox activity (Yin et al., 2015).

All the aforementioned authors (Wang et al., 2013; Yin et al., 2015, 2016) added GO, but Yin et al. (2015, 2016) assumed that it was quickly reduced to RGO by bacterial respiration (Salas et al., 2010). It has been recognised that the use of different reduction routes has a considerable effect on the final RGO properties (e.g. oxidation degree of the final product, chemical structure of the surface) (Alazmi et al., 2016). In this study, chemically reduced RGO was directly added to the anammox biomass. Moreover, all the described studies on the GO/RGO influence on the anammox activity (Wang et al., 2013; Yin et al., 2015, 2016) were conducted only at 35 °C, but at the technological scale it is desired to perform this process at lower temperatures.

All mentioned studies assumed that addition of RGO with proper concentration to the anammox community will increase its activity. However, the research were performed with high temperatures close to the optimum one. The specific activity of anammox bacteria can decrease ten times when the temperature decline from approximately 30 to 10 °C (Lotti et al., 2015b). We assumed that closer look should be carried out on the intensification of anammox activity at low temperatures, and if the anammox is stimulated at temperatures close to optimum it should be also stimulated at lower temperatures. Thus, the main objective of this study was to verify the possibility of supporting the anammox process at low temperatures via an appropriate RGO addition. A central composite design (CCD) was employed to study simultaneous effects of the RGO concentration and low temperatures on the anammox activity.

2. Materials and methods

2.1. Biomass characteristics

The anammox suspended biomass originated from a laboratory-scale sequencing batch reactor (SBR). The reactor was operated at a temperature of 30 ± 1 °C, pH 7.3 ± 0.2, and was fed with a mineral medium with a total nitrogen loading rate of 0.135 ± 0.010 g N/L·d. Mineral medium composition adapted from (van de Graaf et al., 1996): 0.191 g NH_4Cl/L, 0.345 g $NaNO_2$/L, 0.048 g $KHCO_3$/L, 0.041 g KH_2PO_4/L, 0.228 g $MgSO_4 \cdot 7H_2O$/L, 0.007 g $FeSO_4 \cdot 7H_2O$/L, 0.004 g EDTA/L.

2.2. Reduced graphene oxide (RGO) characteristics

The commercially available few-layer reduced graphene oxide used in all the assays was characterised by: bulk density of dry powder: 0.15–0.25 g/mL, Raman spectroscopy result: $I_D/I_G > 1.48$, elemental analysis (percentage by weight): C > 85%, H < 1%, *N* < 3,5%, O < 10%, others ~0,6%, and X-ray fluorescence (XRF) impurities analysis: Cl (0.3%), Mn (0.2%), S (0.01%), K (0.01%), Fe (0.01%), Ca (0.009%), Cu (0.006%), Ni (0.001%).

2.3. Specific anammox activity (SAA) determination

Specific anammox activity was determined in batch tests, according to the methodology described by Tomaszewski et al. (2017b). For the batch tests, biomass samples with the medium were prepared in 125 mL batch anaerobic reactors. A phosphate buffer was added to the batch reactors (to reach a final concentration of 0.14 g KH_2PO_4/L and