



Wydział Inżynierii Środowiska i Energetyki  
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## **STRESZCZENIE ROZPRAWY DOKTORSKIEJ**

**Wspomaganie procesu anammox w niskich temperaturach  
zredukowanym tlenkiem grafenu**

*Supporting the anammox process at low temperatures  
by reduced graphene oxide*

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## ABSTRACT

Anaerobic ammonium oxidation (anammox) process is assumed to be the most efficient method of biological nitrogen removal from wastewater. Its application in the mainstream of the municipal wastewater treatment plant (WWTP) will offer a significant reduction of operating costs and greenhouse gases emission. Unfortunately, bacteria performing anammox process are characterized by low growth rate and relatively high optimal temperature (30 - 40°C), that is one of the main limitations for its implementation in the municipal WWTP. Therefore, the intensification of the anammox activity at temperatures below 20°C (so-called 'cold anammox') has been the focus of recent investigations. Nanomaterials can be an innovative solution to this problem. Several recent studies indicate that the activity and growth rate of the anammox bacteria at 35°C can be accelerated by graphene derivatives. Therefore, it was reasonable to assume that the addition of reduced graphene oxide (RGO) may increase anammox process efficiency at low temperature. Thus, the main objective of this work was to evaluate RGO influence on the anammox process at low temperature (10 - 20°C).

Presented work evaluated: (I) the influence of temperature on the anammox bacteria activity using batch experiments; (II) effect of pH on the anammox activity at low temperatures using batch experiments, planned in accordance with the central composite design (CCD); (III) short-term effects of RGO on the anammox bacteria activity in a wide range of temperature using batch experiments planned in accordance with the CCD; (IV) long-term effects of RGO on the low-temperature anammox process in a sequencing batch reactor (SBR); (V) RGO influence on the bacterial community structure composition by metagenomic approach; (VI) microbial impact on the structure and properties of the RGO, using advanced microscopic and spectroscopic techniques.

Preliminary studies showed that the optimal pH range narrows along with a temperature decrease, which indicates that anammox performance at a low temperature may be supported by more accurate pH control. Proper research indicated that the activity of anammox biomass could be enhanced at low temperatures by RGO. Maximum stimulation of the anammox bacteria activity was observed at 13°C, with the addition of 15 mg/L of RGO. During short- and long-term experiments 28% and 17% stimulation was observed, respectively. Moreover, it was noted that the activity increase tends to be stronger at lower

temperatures ( $< 20^{\circ}\text{C}$ ), which is probably connected with the change in the kinetics of the anammox reaction in these temperatures. Summary of all obtained results allowed to state that RGO effects probably do not depend on the nanomaterial concentration, but on the dose per biomass unit. The optimal dose for the anammox stimulation was evaluated between 20 and 45 mg/g VSS (volatile suspended solids). Microbial analysis showed, that the bacterial community structure was not influenced by the addition of RGO. These results underscore that bacteria living in complex communities seem to have a higher resistance towards external agents such as RGO. Moreover, after incubation in the anammox bioreactor RGO showed signs of degradation and chemical changes. Based on all results analysis, it was proposed that RGO can stimulate anammox activity in two ways: bacteria growth rate stimulation and the increase in the enzymatic reactions rate.

In conclusion, the presented work proved that the addition of RGO can support the efficiency of the anammox process at low temperatures. The interdisciplinary approach included RGO influence on the nitrogen removal, bacterial community structure composition and bacterial impact on the structure of the nanomaterial. Additionally, presented results revealed, that bacteria living in the anammox activated sludge community can degrade and/or modify the structure of the nanomaterial. Since a greater concentration of graphene-based nanomaterials is expected in the wastewater treatment plants, this observation is very promising.