Abstract

Extensive and uncontrolled use of antibiotics in medicine, veterinary, and animal breeding are directly reflected in their occurrence in the environment (especially in the aquatic environment). A few thousand tons of antimicrobials and their transformation products are estimated to be annually worldwide introduced to the environment. Wastewater treatment plants (WWTPs) have been found as the main source of antibiotics in natural waters (surface water, groundwater, etc.). The occurrence of antibiotics in WWTPs is related to the development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs), which pose a worldwide risk to human and animal health. In addition, antibiotics have a negative impact on the process of biological wastewater treatment, including nitrification, and denitrification, for example, causing a decrease in metabolic activity resulting in a reduction in the efficiency of wastewater treatment. One such process that is particularly vulnerable to antibiotics is anammox (anaerobic ammonia oxidation). Anammox is known as an innovative and more sustainable biological rich-nitrogen removal alternative to conventional nitrification-denitrification technology. However, the bacteria that carry out the anammox process are susceptible to toxic substances found in wastewater (including antibiotics), which can make it difficult to implement the process on a technical scale.

The presented doctoral dissertation includes three publications describing the interaction between three popular antibiotics (oxytetracycline (OTC), ciprofloxacin (CIP), and clarithromycin (CLA)) and anammox process and microorganisms which perform it. The research was preceded by a detailed literature analysis (**Publication 1**), which revealed research directions in which currently available knowledge was limited. In particular, **Publication 1** showed a lack of sufficient research on low concentrations of antibiotics (that can be found in wastewater) and a lack of research on antibiotics that are commonly found in wastewater in large quantities, such as ciprofloxacin and clarithromycin. The anammox process was carried out in a sequencing batch reactor (SBR). Experiments evaluated: (I) the effect of trace concentration (0.001 mg L^{-1}) of antibiotics on the anammox process performance, (II) the influence of successive concentration (0.001-100 mg L^{-1}) of antibiotics on the effectiveness of anammox process performance, (III) antibiotics influence on community structure of anammox bacteria in activated sludge, (IV) effect of antibiotics on the abundance of the nitrogen-cycle functional genes in the anammox community, (V) antibiotics impact on the microbial properties and anammox cell structures, (VI) investigating the

protective mechanisms of anammox bacteria against antibiotics based on extracellular polymer substances (EPSs) production and exchange of antibiotic resistance genes (ARGs).

The results presented in **Publication 2** show that the short-term effect of antibiotics at 0.001 mg L^{-1} on the anammox process caused an increase in anammox activity by about 7.1% under OTC pressure while both CIP and CLA caused a decrease in anammox activity by about 8.4% and 3.2%, respectively. The long-term test showed no significant change in the efficiency of the anammox process, which may be due to the protection of anammox bacteria from such low concentrations of antibiotics mainly by the production of EPSs. Nevertheless, each antibiotic caused changes in community structure decreasing the abundance of Candidatus *Brocadia*, while the abundance of *Nitrospira* increased by 1.68% (bioreactor with OTC addition), 4.43% (bioreactor with CIP addition), 3.08% (bioreactor with CLA addition). The presence of *Nitrospira* bacteria may be related to its ability to carry out complete nitrification (comammox) under oxygen-limited conditions. The changes in the community structure corresponded with changes in the abundance of functional genes, where the abundance of the *hzo* gene decreased, while the gene responsible for nitrification (*amoA* and *nxrA*) had a much higher abundance than the *hzo* gene during the whole experiment. Trace concentrations of antibiotics have also caused a development in antibiotic resistance. Among 6 detected genes that determined resistance to tested antibiotics (*tetX*, *tetC*, *tetW* – ARGs-OTC; *mphA* – ARGs-CLA; *qnrB4*, *qnrS* – ARGs-CIP) up to 4 obtained an increase in abundance (*tetW*, *tetC*, *qnrB4*, *qnrS*). **Publication 3** noted that long-term exposure to the antibiotic at concentrations above 1 mg L^{-1} has a significant effect on reducing the efficiency of the anammox process. Correspondingly, the nitrogen removal rate (NRR) decreased by 27% (OTC), 30% (CIP), and 56% (CLA), suspecting that CLA has the most adverse effect on the anammox process. Co-occurrence analysis of community structure and anammox functional genes revealed the development of *Planctomycetes* under each antibiotic's suppression, while probably most of these bacteria were unable to perform of anammox process. Similar to the results presented in publication 2, the abundance of *Nitrospira* increased as the concentration of antibiotics increased. During the experimental period, it was observed the development of antibiotic resistance was almost in all found ARGs determining tested antibiotics. The abundance of each ARGs was higher than its initial level. Additionally, co-occurrence analysis between dominant species, ARGs, and functional genes indicated a significant role for Candidatus *Jettenia* in the transfer of CLA resistance.

In conclusion, the presented dissertation proved that antibiotics in concentrations such as those found in wastewater do not affect the effectiveness of the anammox process. However,

they cause changes in the bacterial community structure of the activated sludge and cause the exchange of resistance genes determining the resistance towards the antibiotics being tested. Moreover, only higher concentrations of antibiotics (above few mg L^{-1}) cause significant decreases in the effectiveness of the anammox process and caused changes in anammox cell structures. Additionally, as tested, bacteria living in the anammox biomass community are able to counteract antibiotics suppression by the production of EPSs and transfer of ARGs. It was proven that Candidtus *Jettenia* participated in the transfer of ARGs determining CLA resistance.