

Understanding the co-selection mechanisms of antibiotic and heavy metal resistance during methane fermentation of sewage sludge - nanopore long-read metagenomic approach

The quantities of sewage sludge produced in wastewater treatment plants continue to increase (millions of tonnes are generated each year around the world). Wastewater contains various pollutants, including antimicrobials and heavy metals, which are accumulated in sewage sludge. Both wastewater and sewage sludge are also massively colonized by microorganisms, including pathogens. Bacteria harboring antibiotic resistance genes (ARGs) and heavy metal resistance genes (MRGs) pose a particular threat. In addition, wastewater and sewage sludge contain subinhibitory concentrations of antimicrobials, which promote increased selection pressure on bacteria harboring specific ARGs. Moreover, anthropogenic heavy metal pollution may impose additional selection pressure on microorganisms and promote bacteria with specific MRGs. The exchange of genetic structures, including ARGs and MRGs, between microorganisms contributes to the spread of antibiotic resistance (AR) and heavy metal resistance (MR).

It is particularly important, that the occurrence of some ARGs may be directly related to the heavy metal presence in the environment. The simultaneous selection of AR and MR is interpreted as co-selection. Co-selection mechanisms include co-resistance (when both ARGs and MRGs are located within the same mobile genetic elements, for example plasmids) and cross-resistance (when one gene simultaneously determines AR and MR). Considering the alarming anthropogenic levels of pollution in the environment, the close linkage between ARGs and MRGs and especially their co-transfer via mobile genetic elements poses the biggest challenge for research concerning microbial resistance. The scientific literature does not provide information on the co-selection of AR and MR during the methane fermentation of sewage sludge.

Methane fermentation is one of the key strategies for stabilizing sewage sludge from wastewater treatment plants. The presence of inhibitors, such as antibiotics and heavy metals, can destabilize this process and decrease methane output. To maximize the efficiency of methane fermentation, it is extremely important to control the pollutants present in the substrate and monitor the processing parameters in reactors.

In this study, sewage sludge will be exposed to antibiotics and heavy metals, having regard to real, environmental concentrations of these pollutants. Sewage sludge as a substrate will be introduced into anaerobic reactors. Antibiotics and heavy metals will be dozen into the bioreactors, both individually and in combination. The results obtained during individual exposure to inhibitors will constitute a reference point in assessing the antibiotic-heavy metal synergistic or antagonistic interaction. The control bioreactor will not be exposed to inhibitors. Based on the preliminary studies and scientific literature, three commonly used antibiotics and two heavy metals have been selected for this project. The efforts put into properly selecting inhibitors make it possible to perceive them as significant factors for the co-selection of AR and MR during methane fermentation of sewage sludge.

The main purpose of this project is to understand the mechanisms of AR and MR co-selection. Primarily, this study will comprehensively determine the impact of antibiotics and heavy metals on the spread of AR and MR during methane fermentation of sewage sludge. Because the genetic context of ARGs, MRGs, and mobile genetic elements is often unknown, this project includes a modern molecular biology tool - nanopore long-read DNA sequencing. Nanopore sequencing has the powerful potential to become a tool for the identification of (1) AR and MR co-selection effect, (2) ARG and MRG mobility, and (3) ARG and MRG hosts. Additionally, the designed studies will enable the effect of inhibitors on the efficiency of the sewage sludge methane fermentation. The methane production, biogas quality, and volatile fatty acids content will be determined. Moreover, the effect of the heavy metal presence on the removal of antimicrobial substances will be analyzed.

The occurrence of ARGs and MRGs in anaerobically digested sewage sludge (which is particularly susceptible to the accumulation of antibiotics and heavy metals) is still poorly understood. Moreover, there are no studies that would specify the impact of these pollutants on the co-selection of AR and MR, including their mechanisms. For this reason, this project will allow obtaining results that are currently not available in the scientific literature. The analyses planned in the project will fill this significant gap and deliver a valuable contribution to the science knowledge. The multidisciplinary approach, including both unique molecular methods and physicochemical analyses, can be considered pioneering. The knowledge gained during its execution will be of interest to experts in such fields as environmental engineering, biotechnology, chemistry, and microbiology.