

## MUNICIPAL WWTP SEEN THROUGH THE PRISM OF THE IRMS METHOD

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Wastewater treatment plants (WWTPs) represent complex engineered bioreactors characterized by steep physicochemical gradients that establish heterogeneous microenvironments, influencing microbial activity and biochemical transformations. Despite significant advancements and widespread implementation of treatment technologies worldwide, major knowledge gaps persist in elucidating the microbiological and biochemical mechanisms governing pollutant fate and transformation. The multifaceted complexity of these processes stems from the diverse interactions within microbial consortia and the dynamic nature of operational and environmental parameters, which complicates a holistic understanding of treatment efficacy.

Isotope Ratio Mass Spectrometry (IRMS) is a paramount analytical technique facilitating high-precision quantification of stable isotope ratios, typically expressed in delta notation ( $\delta$ , ‰) relative to international standards. IRMS commonly targets isotopes of hydrogen ( $^2\text{H}/^1\text{H}$ ), carbon ( $^{13}\text{C}/^{12}\text{C}$ ), nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ), oxygen ( $^{18}\text{O}/^{16}\text{O}$ ), and sulphur ( $^{34}\text{S}/^{32}\text{S}$ ). These isotope ratios are established based on invariant natural baselines forged during Earth's formation but are subsequently influenced by physical, chemical, and biological processes active within WWTPs, such as nitrification, denitrification, ammonification, and organic matter degradation. During these transformations, preferential utilization of lighter isotopes ( $^{12}\text{C}$ ,  $^{14}\text{N}$ ) results in isotopic enrichment of residual substrates with heavier isotopes ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ), providing detailed insights into the metabolic pathways and environmental influences affecting organic matter processing.

Acknowledging that stable isotope ratios serve as sensitive quantitative biomarkers susceptible to fractionation from various biochemical and physicochemical factors, this study also investigates the potential isotopic effects induced by pretreatment procedures applied to sewage sludge prior to further processing. A comprehensive literature review of common sludge pretreatment methods was conducted, focusing on their operational principles and impacts on sludge composition.

Empirical investigations were performed on sludge samples collected from six distinct points within the Hajdów Municipal Wastewater Treatment Plant in Lublin, Poland, which operates at an average daily flow rate of 120,000 m<sup>3</sup>/d. Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in these samples were measured using IRMS to assess the influence of selected pretreatment techniques on isotopic signatures. The results demonstrate that while pretreatment effects on isotopic composition are subtle, they exhibit a complex pattern and can surpass isotopic shifts related to the primary sample treatment processes themselves.

These findings contribute to refining the interpretation of stable isotope data in wastewater research and emphasize the necessity for standardized protocols accounting for pretreatment impacts to improve the understanding of microbial-mediated treatment pathways and pollutant transformations within engineered wastewater systems.