Extracellular Peptidases in Wastewater: Specificity and Potential to Biotransform Antimicrobial Peptides

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Anthropogenic chemicals, encompassing pharmaceuticals and personal care products, are integral components of most people's lifes. Post-use, a substantial fraction of these chemicals enters the wastewater system. If not entirely removed during the wastewater treatment, chemicals are released into the environment, where they can have adverse effects on organisms and ecosystem functions. In the case of antibiotics, there is the risk of the emergence of antibiotic resistance genes, amplifying the challenges associated with antimicrobial resistance. While peptide-based chemicals such as antimicrobial peptides (AMPs) have garnered increasing interest in research and development as well as in clinical settings,[1,2] a detailed understanding of their fate in wastewater and WWTPs remains missing. Wastewater inherently harbours a variety of extracellular peptidases. We hypothesize that these peptidases can hydrolyse certain peptide-based micropollutants, thereby preventing their release into the environment. To delve deeper into the fate of peptide-based chemicals in wastewater, we assessed the peptidase activity at different stages of four full-scale WWTPs. Influent samples consistently exhibited highest extracellular peptidase activities compared to later treatment stages.[3] Incubating a set of ten chemically diverse AMPs with extracellular peptidases from influent samples revealed variable biotransformation extents, yet trends across different WWTPs were consistent. Screening the high-resolution mass spectrometry (HRMS) data for transformation products (TPs) of rapidly degraded AMPs, we detected a distinct set of TPs. This finding highlighted the specificity of extracellular peptidases. When comparing extracts from influents from four different full-scale WWTPs, we discovered that almost the same set of TPs was formed, indicating that the specificity is conserved across different wastewaters.[3] To more comprehensively understand peptidase specificity in wastewater, we employed a systematically designed library of 124 tetra-decapeptides[4] spiked into extracellular wastewater extracts from influent samples and monitored peptide concentrations using UHPLC-HRMS. By analysing the formed transformation products, we were able to determine the specificity of extracellular peptidases in wastewater. Collectively, these findings contribute to a holistic understanding of extracellular peptidases in wastewater, enabling predictions about the fate of peptide-based chemicals. Moreover, these findings have the potential to inform the redesign of compounds or aid in developing novel compounds with enhanced biodegradability in wastewater.

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