Municipal Activated Sludge-Derived Microplastic Microbiomes: The Good, the Bad, and the Promising

Dung Ngoc Pham, Lerone Clark, Jason Ong, and ***Mengyan Li*** (mengyan.li@njit.edu) (New Jersey Institute of Technology, Newark, NJ, USA)

Background/Objectives. Microplastics (<5 mm) in wastewater treatment plants (WWTPs) have received increasing attention, given their imminent threats to aquatic ecosystems and public health. Microplastic particles in personal care and cosmetic products, such as toothpaste and facial wash, are washed down the drain, converging at WWTPs as contamination "hotspots". It is estimated that a median-sized WWTP with an average treatment capacity of 5×10^7 m^3/year can discharge up to 2×10^6 microplastic particles per day. These microplastics perpetually enter inland rivers, estuaries, and other receiving waters that eventually drain to oceans. As a unique microhabitat, microplastics can promote the formation of biofilm, a slimy buildup of interactive and resistant microorganisms on the surface. Molecular analysis of microplastic biofilms discloses a broad spectrum of antibiotic resistance genes (ARGs), conferring sulfonamide (SA), beta-lactam, aminoglycoside, and other antimicrobial resistance. Furthermore, plastisphere promotes horizontal gene transfer (HGT), raising the chance of transmitting ARGs to (opportunistic) pathogenic bacteria that co-reside on microplastics. In this study, we set up microcosms using three different municipal activated sludges and investigated the acclimated plastisphere regarding the shifting of ARG abundances and microbial compositions in response to the exposure to spherical polyethylene (PE) and polystyrene (PS) microplastics. These two types of microplastics were selected considering their wide use in commercial products and prevalent detection in municipal wastewaters. For comparison, control treatments were prepared with fine sands as natural suspended particles abundant in the activated sludge tanks at WWTPs.

Approach/Activities. Activated sludge samples were collected from aeration tanks of three WWTPs (designated as Sludge P, R, and L) located in northern New Jersey in June and October 2019. These WWTPs served resident populations ranging from 6.0×10^4 to 1.4×10^6, as well as a diversity of domestic industries. Thus, activated sludge samples from these three WWTPs were selected as seeding inocula for biological parallels to better represent sludge communities with varying constitutes and concentrations of ARB and pathogens. For each sludge sample, three treatments were prepared in 25-mL glass bottles containing 6 mg of one of the three microparticles (i.e., PE, PS, or sand) and 5 mL of the sludge culture. In addition, parallel treatments were spiked with SMX at an initial concentration of 100 μg/L, representing the relatively high contamination of SMX and other SAs detected in municipal and pharmaceutical wastewater (0.015~1340 μg/L). All treatments were conducted in triplicate.

Results/Lessons Learned. In this batch study with activated sludge samples from three domestic WWTPs, we demonstrated both polyethylene (PE) and polystyrene (PS) microplastics can acclimate biofilms enriched with sulfonamide resistance genes (*sul1* and *sul2*) and the associated mobile genetic element (*intI1*) in comparison with fine sands as control particles. Absolute abundances of these genes were further elevated by 1.2~4.5 fold when sulfamethoxazole was initially spiked as a representative sulfonamide. The combination of 16S rRNA amplicon sequencing and differential ranking analysis revealed that microplastics selectively promoted antibiotic-resistant and pathogenic taxa (e.g., *Raoultella ornithinolytica* and *Stenotrophomonas maltophilia*) with enrichment indices ranging from 1.6 to 3.3. Furthermore, heterotrophic *Novosphingobium* and filamentous *Flectobacillus* accounted for 14.6 % and 3.3 % on average in microplastic biofilms, respectively, which were up to 2.8 and 11.1 times higher

than those in sand biofilms. Dominance of these bacterial species may contribute to initial biofilm formation that facilitates subsequent colonization and proliferation of ARB and pathogens, thus amplifying their risks in the receiving environments and beyond.