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## Divergence of the biofilm architecture, component and microbiome involved in the fouling of membrane bioreactors

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In nature, microbes form complexes called biofilms that comprise cells and extracellular matrixes. In membrane bioreactors (MBRs) for wastewater treatment and reclamation, biofilm formation on the filtration membrane and the subsequent clogging of membrane pores (called biofouling) is one of the most persistent problems. In this regard, sodium hypochlorite (NaOCl) has been generally used to wash clogged membranes for recovery and maintenance purposes. Here, we investigated the structure and microbiome of biofilms formed on filtration membrane in the MBR under several different conditions. The biofilm structure was investigated using non-destructive confocal reflection microscopy (CRM) and high-throughput sequencing of 16S rRNA genes. Direct CRM indicated that the biofilms maintained a state of thinness regardless of the increasing transmembrane pressure (TMP) at low organic loading rates (OLRs). Their components were primarily extracellular polysaccharides and microbial cells. In contrast, high OLRs resulted in a rapid increase in the TMP and the development of thick biofilms mainly composed of extracellular lipids. High-throughput sequencing revealed that the biofilm microbiomes, substantially changed in response to the OLR conditions and biofilm development. Secondary, to evaluate the influence of NaOCl treatment on clogged membranes with biofilm formation, an attenuated total reflection Fourier transform infrared (ATR-FT-IR) spectrometer analysis was performed. ATR-FT-IR indicated the disappearance of functional groups representing different membrane fouling compounds after treatment with NaOCl. However, the high-throughput sequencing revealed the presence of residual halotolerant bacteria even after treatment with NaOCl. These results demonstrated that the architectures, chemical components and microbiomes of the biofilms on fouled membranes were highly diverse, and the formation of biofilms made it difficult to remove the biofouling completely.