

Session: Emerging technologies

Linking adhesion of EPS with the temporal gene expression of microbial biofilms developed in wastewater on RO membranes: Meta-transcriptomic analysis combined with a QCM-D study

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The development of microbial biofilms on reverse osmosis (RO) membranes is a major concern for wastewater effluent desalination, leading to a decrease in the efficiency of the process, and a huge monetary loss to desalination plants. The advanced meta-transcriptome methodology, providing a huge data set of gene expression in natural biofilm consortia and the advanced methodology of physical and mechanical characterization of extracellular polymeric substances (EPS) developed in the last few years, have led us to combine the two approaches and to link between the evolution in EPS adhesion, the involved microbial communities and the physiology (function) of the natural consortia developed on the RO membrane. RO Membranes coupons with microbial biofilm were taken out after 48 and 96 hours of biofilm growth with synthetic tertiary wastewater in the RO flow cell. Extracted RNA was sequenced using NGS techniques and was analyzed using bioinformatic tools. In addition, extracted EPS was studied for its adhesion and viscoelastic properties using quartz crystal microbalance with dissipation monitoring (QCM-D) and biofilm imaging was carried out by scanning electron and confocal laser scanning microscopes (SEM and CLSM, respectively). Initial analysis of the raw sequence reads for meta-transcriptomic analysis was carried out using the Snakemake platform. The sequences were quality trimmed and filtered using Trimmomatic. Taxonomic analysis of the microbiota was based on assembled 16S rRNA transcripts, followed by functional and taxonomic annotation of transcripts derived from the mRNA fraction. SortMeRNA was used to map and filter rRNA sequences. For taxonomic analysis of the mapped reads (rRNA sequences) the single-end mapped reads were analyzed using the phyloFlash pipeline. For functional and taxonomic analysis of the unmapped reads (mRNA sequences), the reads were assembled into transcripts using Genome-Guided Trinity Transcriptome Assembly. Interestingly, in this study we provide a direct link between **(i)** biofilm growth microscopic observations; **(ii)** evolution of EPS adherence; **(iii)** elevation of the EPS shear and viscosity moduli; and lastly and most important: **(iv)** robust changes in the expression of large data set of polysaccharide synthesis relating genes rather than a robust microbial communities analysis. Hence, we highlight the importance of the microbes' function rather than their taxonomy for biofilm structure and integrity on the RO membrane surface.