Quorum Sensing, biofilm formation and interactions among bacteria, yeast and protist isolated from MBR wastewater treatment systems

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Abstract

Social and technical development has aroused the need for new and more efficient wastewater depuration technologies. The membrane bioreactor (MBR) wastewater treatment system is a variant of the conventional activated sludge process, in which the separation of the biomass and the treated water is achieved by filtration rather than by decantation. MBR have several advantages over the conventional activated sludge technology mainly related to the removal of nutrients and pathogenic microorganisms. Membrane fouling is the major inconvenient of MBRs, particularly biofouling which consist on the development of microbial biofilms on the surface of the membrane. *Quorum sensing* is associated with biofouling as it is a regulation mechanism in biofilm formation. The QS is a mechanism of bacterial communication mediated by signal molecules called autoinducers which are secreted by bacteria. Concerning to gram negative bacteria, dominant group in the wastewater, these autoinducers are acyl homoserine lactones (AHLs), and when their concentration reaches a threshold level (quorum level) in the medium results in the biofilm formation over membranes. Since the recognition of biofilms as a microbial phenomenon involved in wastewater treatment, the structure and composition of the microbial populations and their relationships and interactions, are aspects to be explored in depth in MBR systems. Therefore, knowing about microbial community structure and the characteristics of individual microbial groups present in this system is a requisite to solve this phenomenon. The main objective of this research is to understand the factors that are involved in the biofouling of membranes and to obtain information about the microorganisms causing the development of the biofilm and the potential mechanisms of inhibition of this process.

Gram-negative cultivable isolated bacteria were used in a cross-feeding bioassay to determine the production of AHLs, using the AHL reporter strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NT1 pZLR4. The AHL-producing bacterial isolates were identified by molecular methods, sequencing and analysing the 16S rRNA gene. Furthermore, different yeast strains were isolated, cultured and, finally identified by sequencing the D1/D2 region of 26S rDNA. A phylogenetic analysis of the cultivable AHL-producing bacteria and yeast was performed. The isolated bacteria and yeast strains were used to determine their individual capacity of biofilm formation according to the crystal violet method. Those strains which showed high adherence capabilities were selected for microscopic analysis of biofilm development using confocal laser scanning microscopy. Finally, two species of protists were isolated from the activated sludge by micromanipulation and a study was conducted on their ability ingest the selected bacteria and yeast strains using differential interference contrast (DIC) and epifluorescence microscopy. Also, it has been studied the positive or negative influence of protists on the development of bacterial biofilms by adding the protist to the biofilms formed by the selected strains and examined by DIC and epifluorescence microscopy.

In this study we show that most of the bacteria and yeast strains analysed showed strong to moderate ability to adhere to the studied surface. Also, different growth and colonization patterns have been characterized in the formed biofilms, with different complexity levels. Moreover, protists appear to impact on bacterial biofilms and have influence on its morphology, structure and development. Also, it has been observed in this study that since yeast can be ingested by protists they are part of the food chain established in the bioreactor.