AEROBIC GRANULES IN A SEQUENCING BATCH BIOREACTOR UNDER FLUOROQUINOLONE SHOCK LOADINGS

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The growing occurrence of human and veterinary pharmaceuticals in the environment is causing increasing concern. Fluoroquinolones (FQs) are broad-spectrum antibiotics that play an important role in the treatment of serious bacterial infections. Antibiotics can reach wastewater treatment plants (WWTP) from different routes. Domestic effluents are considered the major contributor but effluents from pharmaceutical industries and hospitals are also of great concern. Granular sequencing batch reactors (SBR) constitute a novel biofilm technology for wastewater treatment extremely promising for the treatment of effluents containing toxic compounds. Aerobic granular sludge has several advantages over activated sludge, such as excellent settling properties, high biomass retention, ability to deal with high organic loading rates and to perform simultaneously diverse biological processes, such as Chemical Oxygen Demand (COD), N and P removal.

This study focused on the effect of intermittent and alternating feeding of different FQs, namely Ofloxacin (OFL), Norfloxacin (NOR) and Ciprofloxacin (CPF), on bioreactor performance and diversity of the microbial population. Activated sludge from a municipal WWTP was used as the inoculum for the start-up of the SBR that was operated during 340 days. The aerobic granules grew under aerobic conditions and after ca. 3 months of reactor operation stable granules were observed. Exposure to FQs affected the granular sludge in terms of morphology, causing a decrease in granule size. The granules started to disintegrate and an increase of the solid content in the effluent after exposure to FQs was observed due to wash-out of unstable granules. Also, a decrease in the SBR bed volume was observed during FQs shock loadings.

The effect of the target fluorinated pharmaceuticals on the main biological processes occurring in the granular sludge SBR, such as nitrification and phosphate removal, was evaluated. Ammonium

and nitrite were practically not detected in the treated effluent (maximum concentration of 0.03 and 0.01 mM for NH_4^+-N and NO_2^--N, respectively) indicating that neither ammonia oxidizing bacteria (AOB) nor nitrite oxidizing bacteria (NOB) were inhibited by the presence of the FQs, whereas phosphate removal was affected. The phosphate released into the bulk liquid by the phosphate accumulating organisms (PAO) during the anaerobic feeding period was not completely removed and the levels of phosphate in the bioreactor effluent increased. The organic removal, measured by COD, was not markedly affected by FQ shock loads.

Changes in the bacterial community from aerobic granules related to FQs shock loadings were examined using denaturing gradient electrophoresis (DGGE) of 16S rRNA. The clustering analysis suggested that samples clustered according to the temporal factor. The gradual succession observed in the bacterial assemblage composition was related with the exposure to FQs. Also, the microbial population present in the aerobic granules was also investigated by culture-dependent methods. Several bacterial isolates were retrieved from the granules with predominance of isolates belonging to α- and ɣ-branch of the *Proteobacteria* phylum.

After withdrawal of the FQ compounds in the inlet stream of the reactor, the granules returned to their initial state. After ca. 2 months without FQs exposure, the SBR bed volume was recovered and the solid content at the bioreactor effluent returned to normal levels.