

TITLE: MOLECULAR BIOLOGY ANALYSIS OF ADHERED BIOMASS IN POLYURETHANE FOAM USED AS SUPPORT MATERIAL IN ANAEROBIC PACKED BED REACTOR TO REMOVE SULFAMETHOXAZOLE AND CIPROFLOXACIN FROM DOMESTIC SEWAGE

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ABSTRACT:

Anaerobic sewage treatment finds great applicability in tropical countries like Brazil, as well as being a low cost solution and still generating methane that can be used as fuel. The biofilm formation in anaerobic reactors with adhered biomass may enhance the biodegradation of xenobiotic compounds. The pharmaceuticals sulfamethoxazole (SMX) and ciprofloxacin (CIP) are among the most frequently detected antibiotics in sanitary sewage. Their presence in the environment can result in the development and dissemination of resistance genes in bacteria. This study evaluated the microbial community present in an anaerobic packed bed reactor (APBR) with polyurethane foam as support material. The reactor was inoculated with granular sludge obtained from an UASB bioreactor treating poultry slaughterhouse wastewater. Initially, the reactor was fed with synthetic wastewater composed by sucrose (43), cellulose (42.5), starch (135), meat extract (219), NaHCO₃ (300), KH₂PO₄ (15), NaCl (250), CaCl₂·2H₂O (7), MgCl₂·6H₂O (4.5), anionic surfactant (15), and soybean oil (31) - values in mg.L⁻¹. In the second phase, the reactor was operated with domestic sewage. SMX and CIP concentrations were approximately of 400 ng.L⁻¹. At the end of each operating stage, polyurethane foam samples were withdrawn from the reactor and their biomass was washed and centrifuged. Then, the biomass DNA was extracted using PBS buffer, glass beads, phenol + Tris-buffer and chloroform. DNA purification was performed with kit Illustra GFX PCR DNA and Gel Band Purification from "GE Healthcare". The purified DNA was sent to genome sequencing. The samples were submitted to the bacterial and archaeal assay consisting of: polymerase chain reaction (PCR) and RNA_r 16S sequencing with 515F/806R primers using Illumina MiSeq technology. Genera such as *Methanobacterium*, *Methanosaeta*, *Methanoregula*, *Bacillus*, *Clostridium*, *Treponema* and *Synergistes* were identified with high relative abundance in the samples and are linked to SMX and CIP degradation in previous reported studies. Other genera that stood out were *Methanolinea*, *Lactivibrio*, *Thermanaerovibrio* and *Cytophaga*. APBR was able to remove SMX and CIP with high efficiency (around 70%), and the predominant microbial communities identified in the biofilm could be associated to the biotransformation of these compounds and stable reactor operation over time.

Keywords: Molecular Biology, Fixed Bed Reactor, Sulfamethoxazole, Ciprofloxacin, Poliurethane Foam, Domestic sewage.

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