

**TITLE:** EVALUATION OF THE MICROBIAL COMMUNITY IN ANAEROBIC STRUCTURED BED REACTOR OPERATED WITH DIFFERENT INOCULUM SOURCES FOR SULFAMETOXAZOLE REMOVAL

**AUTHORS:** SELLA, C. F.; CARNEIRO, R.B.; POZZI, E.; SAKAMOTO, I. K.; ZAIAT, M.

**INSTITUTION:** LABORATORY OF BIOLOGICAL PROCESSES, SÃO CARLOS SCHOOL OF ENGINEERING, UNIVERSITY OF SÃO PAULO (USP), 1100, JOÃO DAGNONE AVE., SANTA ANGELINA, 13563-120, SÃO CARLOS, SÃO PAULO, BRAZIL

**ABSTRACT:**

The antibiotic Sulfamethoxazole (SMX) is widely used in veterinary and human medicine and has been detected in small concentrations in wastewater and water supply. This raises questions about bacterial resistance, effects on human health and the search for better ways of removing SMX from sewage. The objective of this study was evaluating the microbial community present in the biofilm of an ASBR (Anaerobic Structured Bed Reactor) operated with three different sources of inoculum with focus on the SMX removal: poultry slaughtering sludge, brewery sludge and sewage treatment plant sludge. The study was divided into three operational stages, each one with a sludge source. The reactor was acclimatized at 30°C and fed with a synthetic substrate simulating a domestic sewage with 400 ng.L<sup>-1</sup> of SMX. For the antibiotic analysis the liquid chromatograph 1200 Infinity (Agilent, USA) was used. For the microbiological assays, the inoculated biomass and the adhered biomass after the operational period were submitted to phase contrast and fluorescence microscopy and denaturing gradient gel electrophoresis (DGGE) for the Archaea and Bacteria Domains. The SMX removal were 90 ± 5%, 84 ± 6% and 66 ± 10% for the poultry, brewery and sewage sludge, respectively (just the third one presented a significant difference over time in T-test with  $\alpha=5\%$ ). In the comparison of the microbial community between the inoculum and the biomass at the end of the operation, for the Bacteria Domain it was 74%, 9% and 9% and for Archaea it was 79%, 88% and 14% for poultry, brewery and sewage, respectively. This indicates that the microbial community that best adapted to the operational condition was poultry for both Archaea and Bacteria Domains. In the microscopic analysis were found organisms like *Methanosaeta* in the poultry and sewage sludge of, which indicates a methanogenic pathway preferentially acetoclastic in the reactor; and the presence of organisms like *Methanosaeta* and like *Methanosarcina* in the brewery sludge, as well as fluorescent bacilli, which indicates the hydrogenotrophic pathway in the reactor. The sewage sludge showed to be more sensitive to the SMX action, confirmed by the lower antibiotic removal. Moreover, the microbial communities of the poultry sludge presented a greater microbial diversity adapted to the SMX.

**Keywords:** Sulfamethoxazole, Microbial communities` selection, Anaerobic fixed bed reactor, DGGE.

**Funding Agency:** São Paulo Research Foundation (FAPESP), Processes nº 2015/06246-7 and 2017/18718-6.