

TITLE: BIOCIDES TOLERANCE IN *Escherichia coli* ISOLATED FROM ANAEROBIC BIODIGESTION SYSTEMS

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ABSTRACT:

Anaerobic digestion is an alternative method for the treatment of animal manure and wastewater. Effluent digesters are known to contain pathogenic enteric microorganisms that may be a risk to human health, as *Escherichia coli*. Thus, the knowledge of the virulence characteristics of clinically relevant bacteria is fundamental for a conscious management of the effluents. The purpose of this work was to evaluate the physiological aspects, such as biocide tolerance profile of *E. coli* isolated from pilot scale anaerobic biodigesters operated at room temperature. In total, 168 *E. coli* strains from the biodigesters were isolated, referred to as the influent (n = 23) and effluent (n = 145), by selective isolation of enterobacteria. Five commercially available biocides commonly used were included in the study. Biocide tolerance was measured by the disk-diffusion technique according to the Clinical and Laboratory Standard Institute guideline with adaptations. Bacterial suspensions were spread on the surface of Mueller Hinton agar plates. A filter paper disc (6mm) was placed and inoculated with 5 µl of the biocide solution. After 24h (35°C incubation), the inhibition halo diameters were measured for influent (IS) and effluent (ES) samples. Test performed in duplicate and Student's t-test was used for evaluation between samples. With statistical difference, the effluent samples showed lower growth halos to the biocides iodopolvidone 10% (IS: 7.9 ± 0.6 mm; ES: 6.8 ± 0.3 mm), sodium hypochlorite 2% (IS: 9.8 ± 0.5 mm; ES: 9.1 ± 0.5 mm), and hydrogen peroxide 3% (IS: 13.8 ± 0.6 mm; ES: 8.1 ± 0.7 mm). For the antiseptic soap (chlorhexidine diglycolate 2%), the influent samples had a statistically lower inhibition halo (IS: 17.6 ± 0.7 mm; ES: 19.0 ± 0.6 mm). No statistical difference (p= 0.205) was observed for o-benzyl p-chlorophenol 0.9% (IS: 7.8 ± 0.5 mm; ES: 7.6 ± 0.4 mm). Biocides are antimicrobial compounds used as disinfectants, antiseptics or preservatives to reduce or eliminate microbial contamination. In this work, we observed that *E. coli* strains were less tolerant to three biocides after anaerobic biodigestion process. An increase in biocide tolerance is an important public health issue which could be expected to contribute to the increased persistence of pathogens. In this sense, the use of biofertilizers in agricultural production systems should be observed microbiological safety for their application.

Keywords: biocides, biofertilizers, dairy cattle manure, microbiological safety.

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