

TITLE: EVALUATION OF THE PHYSIOLOGICAL CHARACTERISTICS ASSOCIATED WITH VIRULENCE IN *Escherichia coli* ISOLATED FROM ANAEROBIC BIODIGESTION SYSTEMS

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ABSTRACT:

Anaerobic digestion figures as a sustainable alternative to avoid discharge of cattle manure in the environment, which results in biogas and biofertilizer. In this regard, with the increasing interest for biogas production and use of effluents on arable land, becomes it import to consider the virulence characteristics of clinically important bacterial populations to sanitary safety and public health management. The purpose of this work was to evaluate the physiological aspects, such as biofilm formation capacity and hemolytic activity, of *Escherichia 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. Hemolytic capacity was evaluated on blood agar supplemented with 5% sheep blood by the formation or not of the halo around the colony. The biofilm formation was evaluated by the crystal-violet staining method on 96-Well polystyrene plates and reading on Elisa reader (596nm filter). As a control, crystal violet binding to wells was measured for wells exposed only to the medium with no bacteria. Student's t-test was used for evaluation between samples. None of the isolated demonstrated hemolytic capacity of the erythrocytes present in the medium. No statistical difference ($p = 0.16$) was observed between the density of stained and aggregated bacterial cells on the surface of the influent (0.229 ± 0.03 nm) and effluent (0.257 ± 0.03 nm) plate. The use of effluents as fertilizer has been discussed as a potential source of infection. In this work, we observed that influent and effluent strains showed the same physiological characteristics, which allows to affirm that the anaerobic digestion process carried out at room temperature did not influence the increase of the virulence capacity of the *E. coli* tested. However, it is important to note that these effluents may contain potentially pathogenic microorganisms capable of causing contamination of soil, water and air. In this sense, the use of biofertilizers from biodigesters in agricultural production systems should be observed microbiological safety for their application.

Keywords: Anaerobic digestion, biofilm, dairy cattle manure, hemolytic activity.

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