



SCREENING OF PROTEASE-PRODUCING MICROORGANISMS FROM ANAEROBIC BIODIGESTION SYSTEM OF DAIRY CATTLE MANURE

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Vol 2, 2022 - 153798

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Abstract

Proteolytic microbial enzymes are biocatalysts with applications in many industrial sectors. Anaerobic digestion is a bioprocess considered a source of protease-producing microorganisms, but it is a little explored environment. In the food industry, microbial enzymes, such as protease, have applicability as additives to improve the nutritional quality of foods and the degradation and extraction of proteins for manufacturing products. This research aimed to screen of protease-producing microorganisms from a Canadian model biodigester operated with dairy cattle manure. Considering the stability of the anaerobic biodigestion process, the samples were collected from the free-stall floor washing water before the solids separator and the biodigester effluent for four weeks in the routine operation. The biodigester was operated at room temperature on a full scale at the experimental farm of Embrapa Dairy Cattle. The pH of the samples was determined using a Tecnal pH meter model, Tec-3MP. The temperature was measured at the conventional metrological station of the National Meteorologic Institute installed in Coronel Pacheco, MG, Brazil. The samples were analyzed by classical microbiology, using the serial dilution method (10-2, 10-3, 10-6, and 10-10) in 0.9% saline solution and by surface scattering (0.1 mL) with the Drigalsky loop help. Samples were grown in duplicate on Skim Milk agar and incubated at 36 °C for 72 hours in aerobic and anaerobic conditions. Protease-producing microorganisms were identified through the formation of a halo around the colonies. Protease-producing microorganisms were cultivated on Brain Heart Infusion agar and later transferred to 500 µL of Brain Heart Infusion broth. After 24 hours of growth, 500 µL of 40 % glycerol was added and the isolates were stored at -20 °C. Twenty-one protease-producing microorganisms were isolated, obtained at 7.26 (± 0.20) pH and 18.33 °C (± 1.5) temperature. The isolation of these microorganisms occurs from 3 to 11 pH range and temperature below 20 °C, which is reinforced by the literature data. The number of isolates

is related to the different characteristics and phases of the bioprocess present at each collection point. Thus, the methodology used proved to be capable of isolating protease-producing microorganisms in an anaerobic biodigestion system of dairy cattle manure.



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Track

- Food biochemistry and biotechnology (BB)

Keywords

Protease

enzymes

screening

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