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Ion exchange expanded bed chromatography for the purification of an extracelular chitosanase from *Bacillus cereus*

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Background

Oligosaccharides have gained considerable interest in the pharmaceutical chemical, food and medical area, due to their biological properties such as the antibacterial [1], antifungal [2], prebiotic, antidiabetic, immunostimulating and antimutagenic activity, acceleration of calcium absorption, recovery of tissue stimulation and activation of plant resistance against insects and pathogen attacks [3], and antitumor functions [4], and thus have been used in agriculture, food and pharmaceutical industries [5]. The enzyme chitosanase is commonly used in the hydrolysis of chitosan. In this work, the ion exchange Streamline DEAE resin was used on an expanded bed system for chitosanase studies purification. In the first step the adsorption characteristic was determined, followed by the comparison between expansion degree using crude unclarified broth and cell-free.

Methods

Bacillus cereus was employed for chitosanase production. The microrganism was grown on TGE broth. The inoculum cultures were grown on a medium containing 3% chitosan, 6% peptone, 0.5% magnesium sulfate heptahydrate and 1% Potassium phosphate dibasic with pH at 7.0. Chitosanases were produced in a 250 mL flask with 50 mL of same inoculums culture medium. The fermentation was carried out with 10% inoculums at 30ºC on a rotary shaker (120rpm for 24 hours). To investigate the best pH for adsorption of the target enzyme, adsorption tests were performed in flasks using different pH 3.0 - 9.0 by the use of de follow buffers: glycine-HCl, sodium acetate, phosphate and carbonate-bicarbonate. The adsorption isotherm and adsorption parameters of the Streamline-DEAE chromatographic matrix were obtained by shake flasks experiments using different dilutions of the crude extract. The flasks were placed in a shaking at 28ºC and 1 mL of Streamline-DEAE was added to each flasks. After 60 minutes of equilibration time the content of the flasks was analyzed. The equilibrium concentration of chitosanases in the bulk liquid phase was determined by quantification of reducing sugars raised and the chitosanase bound per mL of the ion-exchanger adsorbent at equilibrium was calculated by a mass balance. The maximum binding capacity was obtained using the linear form of the Langmuir's equation. To determine the effect of the presence of cells in the bed expansion, the adsorbent was poured to give a settled bed height of 5 cm. The bed expansion characteristic was determined at 28ºC by visually monitoring the bed height as a function of increasing velocity.

Results and conclusions

The adsorption experiments showed that the best pH for adsorption of enzyme dilution was 8.0 and dilution of 50% crude extract was enough to saturate the resin. Data showed a maximum equilibrium binding capacity of 3.74 U/mL and dissociation constant was 0.4 U/mL calculated considering the linear form of the Langmuir's equation. The use of unclarified broth affected drastically the expansion of the bed. Several authors use expansion degree of 2.0, using an unclarified broth was not possible to achieve this expansion while using the cell free broth was possible to achieve the degree of expansion of 2.7.

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