ISOLATION AND CHARACTERIZATION OF ENDOGLUCANASE FROM SEWAGE SLUDGE UNDER BIOSULPHIDOGENIC CONDITIONS OF SEWAGE HYDROLYSIS

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Endoglucanases play a key role in cellulose degradation and catalyze the initial attack on the polymer by hydrolyzing the β-1,4 glucosidic bonds within the amorphous regions of cellulose chains. Cellulolytic bacteria have been isolated and characterized from the sewage sludge and the activation of several hydrolytic enzymes under biosulphidogenic conditions of sewage hydrolysis have been reported. The aims of this study were to isolate, perform the physico-chemical characterization of endoglucanase from sewage sludge under sulphate reduction conditions of sewage hydrolysis and to investigate its $V_{\text{max,app}}$ and $K_{\text{m,app}}$ values using different substrates. The membrane associated endoglucanase activities were shown to have pH optima of 6 and temperature optima of 50°C. The enzymes were thermally more stable when immobilized to the floc matrix of the sludge than when they were released into the aqueous solution via sonication. For both immobilized and released enzymes, sulphate was slightly inhibitory, activity was reduced to 84 % and 77.5 % of the initial activity at sulphate concentrations between 200 and 1000 mg.ml$^{-1}$, respectively. Sulphite was stimulatory to the immobilized enzymes between 200 and 800 mg.ml$^{-1}$. Sulphide stimulated the activities of the endoglucanases, but inhibited activities of the soluble enzymes above 200 mg.ml$^{-1}$. These results were similar to those obtained with other enzymes (lipases, β-glucosidases and proteases) previously studied in the sewage sludge in our research group. The enzyme fraction did not hydrolyse avicel (a crystalline substrate), indicating the absence of any exocellulase activity. For CMC (carboxymethylcellulose) and HEC (hydroxyethylcellulose) the enzyme had $K_{\text{m, app}}$ values of 4 and 5.1 mg.ml$^{-1}$ respectively and $V_{\text{max, app}}$ values of 0.297 and 0.185 µmol glucose min$^{-1}$ml$^{-1}$ respectively. Divalent ions (Cu$^{++}$, Ni$^{++}$ and Zn$^{++}$) proved to be inhibitory while Fe$^{++}$ stimulated the enzyme at concentrations between 200 and 600 mg.ml$^{-1}$. Both Mg$^{++}$ and Ca$^{++}$ were stimulatory at concentrations between 200 and 1000 mg.ml$^{-1}$. All the volatile fatty acids (acetic acid, butyric acid, propionic acid and valeric acid) inhibited the enzymes, with acetic acid eliciting the highest degree of inhibition.

Induction and purification of the enzyme from sewage sludge is currently being conducted, as the sewage sludge might provide a readily available and cheap source of the enzyme for industrial and other uses.