QUANTIFICATION OF ENZYME ACTIVITIES INVOLVED IN CELLULOSE DECOMPOSITION BY A THERMOTOLERANT, MICROAEROPHILIC BACTERIUM GROWN ON DIFFERENT CARBON SOURCES IN BROTH CULTURE

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ABSTRACT

Quantification of microbial enzymes involved in cellulose decomposition is often determined through high pressure liquid chromatography (HPLC), where enzyme activity is determined based on the amount of substrate remaining in the culture. In this study, enzyme activities were determined directly by adding culture medium to additional substrate and measuring the amount of glucose released. Carboxymethylcellulose (CMC), fibrous cellulose, cellobiose, and glucose were used as sole carbon sources to grow a microaerophilic, gram positive bacterial strain at 50 °C. Broth cultures were screened for enzymatic activity over an eight-day period. Every other day, colony forming units (CFU) per ml, pH, sugar concentration and enzyme activity of each broth culture were determined. The enzymatic release of glucose was calculated through comparison to a standard curve using the Somogyi-Nelson colorimetric assay. Glucose concentration and endo-β-1,4-glucanase activity increased over time with the CMC and fibrous cellulose substrates. The amount of glucose released from enzymes in the CMC was 23 times that of the fibrous cellulose samples. A large drop in pH of the cellobiose and glucose samples coincided with lower CFU/ml compared to cultures that did not have such a pH decline. No observable endo-β-1,4-glucanase was detected from either the cellobiose or glucose samples. The data suggest that the optimal time period for enzymatic production is four to six days after inoculation. Further studies will focus on extraction, purification, and quantification of the released cellulase enzymes of this bacterial strain.