ENZYME PRODUCTION IN SUBMERGED FERMENTATION BY ASPERGILLUS NIDULANS

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Abstract
The planet has limited resources of farmland nutrients and fossil energy. Traffic emissions will have to be reduced significantly in the coming years to help abate climate change. In these days, when humankind must face these problems, biogas is considered as one of the most important natural energy sources. Plant biomass is the largest reservoir of environmentally friendly renewable energy on Earth (1-3). However, the complex and recalcitrant structure of the lignocellulose-rich substrates is a severe limitation of biogas production. Agro-industrial processes produce large quantities of corn stalk and wheat straw as plant-waste materials each year. The production of cellulase has been reported from a wide variety of bacteria and fungi. *Aspergillus nidulans* was isolated from cattle rumen under anaerobic conditions. The extracellular enzymes of *A. nidulans* were well represented in the culture media because the specific activity of endoglucanase and β-glucosidase was high.

Introduction
Filamentous fungi are preferred for commercial enzyme production, because the level of the enzymes produced by these cultures is higher than those obtained from bacteria. Almost all fungi of genus *Aspergillus* synthesize cellulase (4), therefore this genus has the potential to dominate the enzyme industry. Industrially important enzymes have traditionally been obtained from submerged fermentation (SmF) because of the ease of handling and greater control of environmental factors such as temperature and pH. There are several articles describing use of agro industrial residues for the production of cellulose such as wheat straw, wheat bran and rice straw as substrates for fungi growth. From this point of view, the organism was isolated from cattle rumen and demonstrated for its improved efficiency in SmF for the production of cellulase using agro-industrial waste as raw material.

Experimental
Aspergillus nidulans was isolated from cattle rumen (Fábiánsebestyén, Hungary) under anaerobic conditions. The isolate was grown on CMC agar medium. The isolated fungal colony was subcultured and maintained on Czapek-Dox-agar plates and stored at 4°C in a refrigerator, until needed. The cellulosic substrates such as corn stalk and wheat straw were chopped to 3-4 mm pieces. The inoculum was prepared by growing the organism in 250 ml Erlenmeyer flask with 50 ml of Czapek-Dox broth containing 30 g/l of α-cellulose, 3 g/l $NaNO_3$, 1 g/l K_2HPO_4, 0.5 g/l $MgSO_4$, 0.5 g/l KCl, 0.5 g/l $FeSO_4$, 15 g/l agar. The medium was inoculated from the Czapek-Dox agar plates and incubated at 37°C for 3 days in a shaker (200 rpm) before it was used for the fermentation process.

Enzyme activity

Assay of *endo-(1,4)-β-D-glucanase* activity was conducted using 3,5-dinitrosalicylic acid (DNS) method by using carboxymethyl cellulose (CMC) as a specific substrate (5).
Measurement of *β-glucocidase* activity was assayed using *p*NPG method (6).

Results and discussion

Cellulase enzymes of *Aspergillus sp.* have traditionally been obtained from submerged fermentation. Microscopic observations of enrichment cultures revealed cellulose fibers, which presumably were released from corn or wheat straw disintegrated by cellulolytic enzymes, surrounded by fungal hyphae (Fig.1.).

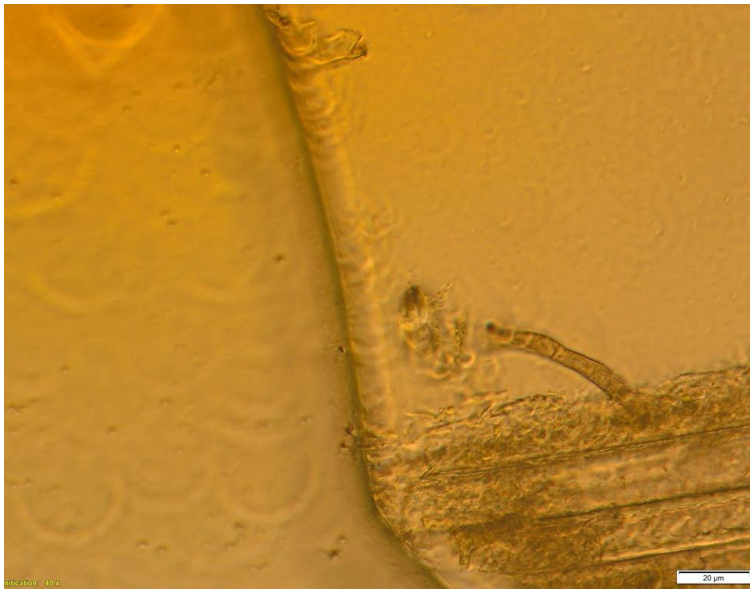

Figure 1. Cellulose fiber surrounded by fungal hyphae

It was established that an optimal inoculum concentration could increase significantly the cellulase production in submerged fermentation. With an increase in substrate concentration from 5 to 25% a rapid growth of fungi was observed which occurred together with increase of enzyme activity. 15-20% substrate concentration was found optimal. Higher or lower substrate ratio resulted in a significant decrease in endoglucanase production (Fig.2). In contrast, β-glucosidase activity showed constant increase between the lowest and the highest concentrations.

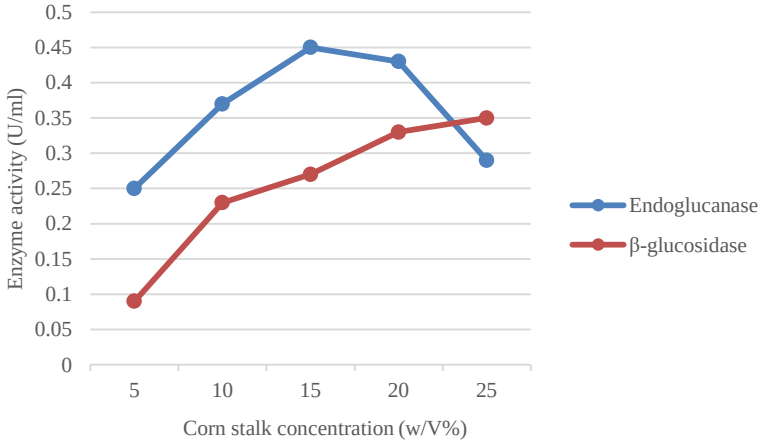

Figure 2. Effect of substrate concentration on cellulose production

Among the tested substrates, the cultivation of the fungus *A. nidulans* on α-cellulose and corn stalk provided the highest endoglucanase and β-glucosidase production (Fig.3.).

Carbon source	Enzyme activity (U/ml)	
	Endoglucanase	β-glucosidase
CMC	0.11	0.02
α-cellulose	0.23	0.45
Microcrystalline cellulose	0.10	0.02
Corn stalk	0.21	0.35
Wheat straw	0.19	0.28

Figure 3. Endoglucanase and β-glucosidase production in different laboratory and agroindustrial substrates

Conclusion

Our results indicated that the extracellular enzymes of *A. nidulans* were well represented in the culture media because the specific activity of endoglucanase and β-glucosidase was high. This result is promising because β-glucosidase is essential for the increased production of glucose from cellulosic substrates as it acts on oligosaccharides and dimers derived from the initial hydrolysis of cellulases and releases monomers from reducing sugars. Test results exploiting the stability of endoglucanases and β-glucosidases at the optimum temperature of biogas fermentation will be presented.

Acknowledgements

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