APPLICATION OF SOLID-STATE FERMENTATION FOR CELLULASE ENZYME PRODUCTION USING *TRICHODERMA VIRIDE*

KIRO MOJSOV

Technological-Technical Faculty
University "Goce Delcev" Stip, Macedonia

UDC: 579.66; 663.15 **JEL Classifications:** L65

Key words: Solid-state fermentation, *Trichoderma viride,* cellulase enzyme, wheat straw agro-waste, single cell protein.

Abstract: The Solid-state fermentation (SSF) is alternative to submerged fermentation for production antibiotics, single cell protein, enzymes, organic acids, biofuel, etc. However, the advantages of SSF in various processes are found to be greater than in submerged fermentation. This technique not only decreases the cost of the process but also makes product cheaper for consumers. The paper describes experimental applicati;on of SSF on wheat straw for production of mycelia protein and cellulase enzymes by *Trichoderma viride.* This actual waste from agriculture industry was used as a nourishing base by *Trichoderma viride* in SSF for cellulase enzyme production. Growth and enzymes production by *Trichoderma viride* were evaluated on wheat straw and alkali treatment wheat straw (wet processing). The growth of the microorganism (biomass content) shows maximum (123.44 mg/petri dish) on alkali treatment wheat straw compared (96.36 mg/petri dish) on wheat straw during of 240 hours. The results obtained demonstrate that the wheat straw waste from agriculture industry can be used as inexpensive base (carbon source) for industrial production on cellulase enzymes by *Trichoderma viride.*

ISSN: 1804-0527 (online) 1804-0519 (print) PP. 108-110

Introduction

For solid-state fermentation (SSF) processes, different agro-industrial wastes are used as solid substrates. Selection of agro-industrial residues for utilization in SSF depends on some physical parameters such as particle size, moisture level, intra-particle spacing and nutrient composition within the substrate. In recent years, some important agro-industrial residues such as sugarcane bagasse, sugar beat pulp/husk, orange bagasse, oil cakes, apple pomace, grape juice, grape seed, coffee husk, wheat bran, cereals, straw, leaves, corncobs etc. have been used as substrates for solid-state fermentation.

In most parts of the country, these materials are mainly used as animal feeds. A large quantity is left on farmlands to be decomposed by microorganism such as bacteria and fungi (Okafor, 1987; Pandey et al., 2000).

Substrates such as agro-industrial residues are proved by many researchers to be better for filamentous fungi. The morphology of filamentous fungi supports them to penetrate the hardest surface due to the presence of turgid pressure at the tip of their mycelium. Hence, the raw materials considered as waste are used for production of value added fine products and reducing pollution problems (Raimbault, 1998).

Enzyme production is one of the most important applications of SSF. SSF has advantages over submerged fermentation (SMF) such as high volumetric productivity, low cost of equipment involved, better yield of product, lesser waste generation and lesser time consuming processes etc.

The type of strain, culture conditions, nature of the substrate and availability of nutrients are the other important factors affecting yield of enzyme production (Pandey et al., 2001). It is crucial to provide optimized water content and control the water activity for good enzyme production. Agro-industrial substrates are considered best for enzyme production in SSF. The cost of enzyme production by submerged fermentation is higher compared to SSF. Tengerdy (1998) have also proved this by comparing cellulase production costs in SSF and SMF. Cellulase production by bacterial strain *Bacillus subtilis* on banana fruit stalk wastes was 12 times higher in SSF than in submerged fermentation under similar experimental condition (Krishna, 1999).

Water content of substrate and aeration rate are critical factors in cellulase production using SSF. Corncob residue was used for cellulase production with *Trichoderma reesei* ZU02 in shallow tray fermentors. Xia and Cen (1999) used a deep trough fermentor with forced aeration for cellulase production. Forced Aeration enhanced the mass transfer to a greater extent, which increased cellulase activity to 305 IU per g of cellulose. It has been reported by Fujian et al. (2002) that substrates in solid-state with continuous circulation of air and convective diffusion with pressure are better for fungal propagation than static cultures. This periodic air circulation increases the looseness of substrates and enhances cellulase activity. The work was performed using steam-exploded wheat straw as carrier with *Penicillum decumbens* in SSF. However, changes in the amplitude of air pressure increased the oxygen availabity to the cultures used and heat removal. The variations enhanced the cellulase production by *Trichoderma viride* in SSF (Tao et al., 1999). However, some newly developed agro-industrial wastes used for cellulase production are banana wastes, rice straw, corn cob residue, rice husk, wheat straw, banana fruit stalk, and coconut coir pith.

Cellulase is an enzyme complex used for the conversion of lignocellulosic residues and used for production of ethanol, single-cell protein, bleaching of pulp, for treatment of waste papers and for fruit juice extraction. In SSF, using lignocellulosic wastes as substrates can reduce the cost of cellulase production (Xia and Cen,1999). The aim of this work was to investigate the effects of application of SSF on wheat straw for production of mycelia protein and cellulase enzymes by *Trichoderma viride.* Growth and enzymes production by *Trichoderma viride* were evaluated on wheat straw and alkali treatment wheat straw (wet processing).

Materials and methods

Micro-organism. The microorganism used in this work was the fungus *Trichoderma viride*, which was isolated

from soil as a highly active producer of cellulase enzymes and was maintained on malt agar slant at 4-8 ^{o}C. Spores from 2 days old agar slants on 28 ^{o}C were collected by adding sterile distilled water to each slant. The spores suspension was counted and diluted to final concentration of 4 . 10^6 spores mL^{-1}.

Substrate. The substrate used was wheat straw and alkali treatment wheat straw (wet processing) which was pick from farmlands after harvest. The substrate was dry milled to 0.5 mm size.

Culture conditions and enzyme production. Aplication is SSF which offers greatest possibilities when fungi are used. Unlike other microorganisms, fungi typically grow in nature on solid substrates. In SSF, the moisture necessary for microbial growth exists in an absorbed state or in complex with solid matrix.

The growth of the microorganism and synthesis of cellulase enzymes were performed in sterilized petri dishes (ø16 mm), with 5 g wheat straw or 5 g alkali treatment wheat straw and 12.5 ml nourishing salts (~ 70% moisture) at 28 ^{o}C within 264 h. As the nourishing salts is used : $(NH_4)_2SO_4$, 0.7 g L^{-1} ; KH_2PO_4,2.0 g L^{-1} ; Urea, 0.3 g L^{-1} ;$CaCl_2$, 0.3 g L^{-1} ; $MgSO_4.7H_2O$, 0.3 g L^{-1}; and Pepton,1.0 g L^{-1}; 1 mL mikroelements. Content of microelements is: $MnSO_4.H_2O$, 1.56 g L^{-1}; $FeSO_4.7\ H_2O$, 5.00 g L^{-1}; $ZnCl_2$, 1.67 g L^{-1} ; and $CoCl_2$, 2.00 g L^{-1}. The initial pH on base, 5. The base was previously sterilized by autoclaving at 121 ^{o}C for 30 min.

Enzyme assay. Filter paper activity (FPA) for total cellulase activity in the cultural filtrate was determined with added Whatman No.1 filter paper strip (1X6 cm;50 mg) immersed in one mililitre of 0.05 M sodium citrate buffer of pH 5.0 and aliquots of suitably diluted filtrate. After incubation at 50 ^{o}C for 1 h, the reducing sugar released was estimated by Somogyi method. One unit (U) of filter paper (FPA) activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar from filter paper per mL per min.

Endoglucanase activity (carboxymethylcellulase; CMCase) was measured as using a reaction mixture containing 1 mL of 1% carboxymethyl cellulose (CMC) in 0.05 M citrate acetate buffer (pH 5.0) and aliquots of suitably diluted filtrate. The reaction mixture was incubated at 50 ^{o}C for 1 h and the reducing sugar produced was determined by Somogyi method. One unit (U) of endoglucanase activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar per mL per min.

Biomass production measurements

Biomass production was measured as dry weight (mg). After filtering, the substrate and the retained cell mass was dried at 70 ^{o}C in an oven until constant weight and then measured. The content of protein in base was estimated according to method of Keldhal [mg biomass = mg protein X 2].

Results and discussion

Solid State Fermentation (SSF) is applied for the processes in which insoluble materials in water are used for the microbial growth (Moo-Young et al.,1983). Water is essential for the microbial growth and in SSF and it is present in thin layers and in occasions, absorbed inside the substrates (Mudgett, 1986).

TABLE 1. GROWTH, TOTAL CELLULASE ACTIVITY (FPA), AND CARBOXYMETHYLCELLULASE ACTIVITY (CMCASE) BY *TRICHODERMA VIRIDE* ON WHEAT STRAW

Fermentation time, h	Dry weight (substrate+biomass), g/petri dish	Protein content, mg/petri dish	Biomass content, mg/petri dish	Substrate, %	FPA, U/mL	CMC U/mL
0	4.358	50.37	0.00	100.0	0.0	0.0
72	4.218	69.99	39.10	95.8	0.640	1.555
96	4.137	75.54	50.32	93.7	0.740	1.844
120	3.950	78.72	56.68	89.3	0.814	2.033
144	3.853	83.26	65.76	86.9	0.888	2.222
168	3.776	87.93	75.10	84.9	0.592	1.405
192	3.730	93.10	85.44	83.6	0.147	0.888
240	3.700	98.56	96.36	82.7	0.073	0.592

Values represented in the table are averages of results of two separately conducted experiments.

TABLE 2. GROWTH, TOTAL CELLULASE ACTIVITY (FPA), AND CARBOXYMETHYLCELLULASE ACTIVITY (CMCASE) BY *TRICHODERMA VIRIDE* ON ALKALI TREATMENT WHEAT STRAW

Fermentation time, h	Dry weight (substrate+biomass), g/petri dish	Protein content, mg/petri dish	Biomass content, mg/petri dish	Substrate, %	FPA, U/mL	CMC U/mL
0	4.328	50.37	0.00	100.0	0.0	0.0
72	4.033	73.64	46.52	92.1	0.073	0.517
96	3.977	85.94	71.14	90.2	0.666	0.814
120	3.892	90.63	80.51	88.0	0.740	0.962
144	3.882	96.90	93.04	87.5	0.407	0.666
168	3.861	102.84	104.94	86.7	0.358	0.517
192	3.750	106.17	115.58	83.9	0.147	0.147
240	3.738	112.09	123.44	83.5	0.073	0.147

Values represented in the table are averages of results of two separately conducted experiments.

During these experiments results shows the extent of production of cellulase enzyme for a period of 120 to 144 hours measured as enzyme activity. Carboxymethylcellulase (CMCase) was maximum (2.222 U/mL) at 144 hours for wheat straw (Table 1), compared with maximum (0.962 U/mL) at 120 hours for alkali treatment wheat straw (wet processing)(Table 2). Filter paper activity (FPA) was maximum (0.888 U/mL) at 144 hours for wheat straw (Table 1), compared with maximum (0.740 U/mL) at 120 hours for alkali treatment wheat straw (Table 2).

The growth of the microorganism (biomass content) shows maximum (123.44 mg/petri dish) on alkali treatment wheat straw (Table 2) compared (96.36 mg/petri dish) on wheat straw (Table 1) during of 240 hours.

Conclusion

Agricultural waste in the form of cellulose which is the most abundant renewable biomass in the biosphere has been shown to be used in the production of valuable products by microorganism. Wheat straw and alkali treatment wheat straw are some of these agricultural wastes used in this work as fermentation substrate which produced a large amount of cellulase enzymes by *Trichoderma viride*. These results highlight the industrial potentials of the substrates as possible raw materials for cellulase enzyme production by *Trichoderma viride*.

References

Fujian, X., Hongzhang, C., Zuohu, L., 2002. "Effect of periodically dynamic changes of air on cellulase oroduction in solid-state fermentation," Enzyme and Microbial Technology, Vol.30, pp.45-48.

Krishna, C., 1999. "Production of bacterial cellulase by sold state bioprocessing of banana wastes," Bioresource Technology, Vol.69, pp. 231-239.

Moo-Young, M., Moreira, A. and Tengerdy, R., 1983. "Principles of solid state fermentation," in: Smith, J., Berry, D., and Kristiansen, B. (Eds.), "Fungal Biotechnology," Edward Arnold Publishers, London, pp.117-144.

Mudgett, R.,1986. "Manual of industrial microbiology and biotechnology", in: Demain, A., and Solomon, N., (Eds.), "American Society for Microbiology," Washington, pp. 66-83.

Okafor, N., 1987. Industrial microbiology, University of Ife Press Ltd., Ile-Ife, Nigeria, pp.32-33.

Pandey, A., Soccol, C., Nigam, P., Soccol, V., 2000. "Biotechnological potential of agro-industrial residues, I: Sugarcane bagasse," Bioresource Technology, Vol.74, pp.69-80.

Pandey, A., Soccol, C., Rodriguez-Leon, J., Nigam, P., 2001. In: "Solid-state fermentation in biotechnology-fundamentals and applications", Asiatech Publ. Inc., New Delhi, pp.50-225.

Raimbault, M., 1998. "General and microbiological aspects of solid substrate fermentation," Electronic Journal of Biotechnology, Vol.1, pp.1-20.

Tao, S., Beihui, L., Zuohu, L., Deming, L., 1999. "Effects of pressure amplitude on cellulase production by *Trichoderma viride* SL-1 in periodic pressure solid state fermenter," Vol.34, pp. 25-29.

Tengerdy, R., 1998. "Solid substrate fermentation for enzyme production," in: Pandey, A. (Ed.), "Advances in Biotechnology," Educational Publ. and Distributors, IP Ext., New Delhi, pp.13-16.

Xia, L., Cen, P., 1999. "Cellulase production by solid state fermentation from the xylose industry," Process Biochemistry, Vol.34, pp.909-912.