RESEARCH PAPER:

Statistical optimization of endoglucanase enzyme production by a local isolate, *Aspergillus heteromorphus* using response surface methodology

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SUMMARY

The production of endoglucanase enzymes by the local isolate of the fungus, *Aspergillus heteromorphus* was investigated. The fungus was cultivated under solid state fermentation condition at 30 0C for 120 hours and endoglucanase production was studied. The effect of initial pH, sugarcane bagasse as substrate and peptone concentration as nitrogen source were optimized using Box- Behnken design as independent variables. The optimal level of each parameter for maximal endoglucanase production by the fungus was determined. Endoglucanase activity was positively influenced by linear increase of peptone concentration and decrease at axial concentration of peptone, bagasse and pH. Results showed that endoglucanase activity was highest 61.7 U/ml when the peptone conc. was 1.75 g/l, bagasse conc. 2.5 g/l and pH was 5 respectively. Validation of predicted results was done, and the experimental values correlated well with that of the predicted.

Key words : *Aspergillus heteromorphus*, Endoglucanase, Sugarcane bagasse, Box-Behnken, Solid-state fermentation

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Due to dwindling of fossil fuels, national security, long- term economic and environmental problems are motivating the continuous search for new and sustainable source of energy. Liquid fuels for transportation can be derived from lignocellulosic biomass by enzymatic hydrolysis and subsequent fermentation to produce bioethanol (Lynd *et al.*, 1999). Enzymatic hydrolysis of cellulosic biomass is considered as the most efficient and least polluting methods for generating glucose from lignocellulosics, but the production economics of bioethanol is largely dependent on the cost of cellulases (Reith *et al.*, 2002). Cellulase is a deceptively complex concept, a convenient shorthand term for four enzyme activities and molecular entities, required for the complete hydrolytic breakdown of macromolecular cellulose to glucose : Endoglucanases, Cellodextrinases, Cellobiohydrolaese and finally β- glocosidases (Singh *et al.*, 2006). The use of agro-industrial residues as the basis for cultivation media is a matter of great interest, aiming to decrease the costs of enzyme production and meeting the increase in awareness on energy conservation and recycling. In the present study, sugarcane bagasse was used as a substrate for enzyme production. Sugarcane converts approximately 2 % of solar energy into chemical bonds of carbohydrates where in two third of these carbohydtates are in the form of lignocelluloses (Singh *et al.*, 2008). Sugarcane contains 12-17% total sugars of which 90% is saccharose and 10% glucose and fructose. Milling extracts roughly 95% of the cane's sugar content leaving behind the solid cane fibre is known as "bagasse" (Wheals, 1999). The amount of bagasse produced in India is 67 million tons in 2006. Sugar cane bagasse is a low-cost and abundant biomass material containing about 27–54% cellulose, which can serve as a potent substrate for cellulase production. There have been several studies on the use of sugarcane bagasse as a substrate in cellulose production both under submerged and SSF (Aiello *et al.*, 1996; Pandey *et al.*, 2000). In order to obtain optimum yield of an enzyme, development of a suitable medium and cultural conditions is obligatory. Selection of appropirate carbon, nitrogen and other nutrients is one of the most critical stages in the development of an efficient and economic bioprocess. Statistical optimization not only allows quick screening of a large experimental domain, but also reflects the role of each of the components. Response surface methodology (RSM) is a powerful tool for the optimization of chemical reactions and industrial processes (Domingos *et al.*, 2008). Response surface methodology (RSM) is a set of useful models for studying the effects of several factors affecting the responses by