Production of cellulolytic enzymes by *Aspergillus fumigatus* ABK9 in wheat bran-rice straw mixed substrate and use of cocktail enzymes for deinking of waste office paper pulp


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**Highlights**

- Solid state mixed substrate fermentation using the ligno-cellulolytic *Aspergillus fumigatus* strain ABK9.
- Optimization of cellulases production through Plackett–Burman and Box–Behnken methodology.
- Enzymatic deinking of waste office paper pulp and comparison with chemical treatment.

**Abstract**

Response surface methodology was employed to optimize mixed substrate solid state fermentation for the production of cellulases and xylanase by *Aspergillus fumigatus* ABK9. Among 11 different parameters, fermentation time (86–88 h), medium pH (6.1–6.2), substrate amount (10.0–10.5 g) and substrate ratio (wheat bran:rice straw) (1:1) had significantly influences on enzyme production. Under these conditions endoglucanase, *β*-glucosidase, FPase (filter paper degrading activity) and xylanase activities of 826.2, 255.16, 102.5 and 1130.4 U/g, respectively were obtained. The enzyme cocktail extracted (solid to water ratio of 1:10) from the ferments increased brightness of waste office paper pulp by 82.8% ISO, InkD value by 82.1%, removed chromophores (2.53 OD; A237 nm) and hydrophobic compounds (1.15 OD; A465 nm) and also decreased the kappa number to 13.5 from 16.8.

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**1. Introduction**

Cellulase enzyme system include endo-β-D-glucanase (EC 3.2.1.4) or endoglucanase, which hydrolyze internal β-1,4-glycosidic bonds randomly; cellobiohydrolase (CBH) (EC.3.2.1.91), which moves progressively along the cellulose chain and cleaves off cellobiose units from the ends and β-glucosidase (EC.3.2.1.21), which converts cellobiose and soluble celloextrinsics into glucose. Xylanases (1,4-β-xylan xylanohydrolase; EC 3.2.1.8) are also important enzymes that help in the degradation of xylan, the most abundant hemicellulose present in both hardwoods and annual plants and accounting for nearly 20–35% of the total dry weight in tropical biomass (Kar et al., 2008; Maity et al., 2012; Mandal et al., 2011). All these enzymes work synergistically to hydrolyze cellulose (Ahamed and Vermette, 2008). Cellulases have already found numerous applications (Hong et al., 2001; Das and Ghosh, 2009). Cellulolytic and hemicellulolytic enzymes can also be employed for deinking of waste office paper for production of chlorine-free pulp (Jurasek and Paice, 1986).

Extensive research is being done on improving the yield of cellulases and xylanases production which involves screening of new enzyme-producing microorganisms, random mutagenesis of fungal strains and genetic engineering of individual enzymes (Jorgensen et al., 2007). Optimization of mixed substrate fermentation with cheap suitable solid substrates in a particular ratio is also a strategy for improvement of enzyme production (Das and Ghosh, 2009). Mixtures of natural substrates provide adequate nutrients and serve as habitat for growth of microorganisms (Rao et al., 2007).

Several statistical methods are available for process optimization (Teng and Xu, 2008). Plackett–Burman statistical method is one such approach involving a two-level fractional factorial