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ORIGINAL ARTICLE

Production of crude enzyme from *Aspergillus nidulans* AKB-25 using black gram residue as the substrate and its industrial applications

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Abstract The production of crop residues in India is estimated to be about 500–550 million tons annually. It is estimated that about 93 million tons of crop residues is burnt annually which is not only wastage of valuable biomass resources but pollution of the environment with the production of green house gases also. Among different low cost crop residues, black gram residue as the substrate produced maximal endoglucanase, FPase, and β-glucosidase activities from *Aspergillus nidulans* AKB-25 under solid-state fermentation. During optimisation of cultural parameters *A. nidulans* AKB-25 produced maximal endoglucanase (152.14 IU/gds), FPase (3.42 FPU/gds) and xylanase (2441.03 IU/gds) activities. The crude enzyme was found effective for the saccharification of pearl millet stover and bio-deinking of mixed office waste paper. The crude enzyme from *A. nidulans* AKB-25 produced maximum fermentable sugars of 546.91 mg/g from alkali-pretreated pearl millet stover by saccharification process at a dose of 15 FPU/g of substrate. Pulp brightness and deinking efficiency of mixed office waste paper improved by 4.6% and 25.01% respectively and mitigated dirt counts by 74.70% after bio-deinking. Physical strength properties like burst index, tensile index and double fold number were also improved during bio-deinking of mixed office waste paper.

1. Introduction

India is primarily an agrarian country and one-third of the national income comes from agriculture. The production of crop residues in India is estimated to be about 500–550 million tons annually. The crop residues covers the whole range of biomass produced as by-products from growing and processing crops and it encompasses all agricultural wastes such as sugarcane bagasse, straw, stem, stalk, leaves, husk, shell, peel, pulp, stubble, etc. It is estimated that about 93 million tons of crop residues left in the field are burnt annually [1]. The crop residues are partially dried in the fields and a fraction is burnt to generate steam for the stripping; the rest is left in the fields where natural biodegradation takes place. The burning of crop residues is not only the wastage of valuable biomass resources which could be a source of carbon, bioactive compounds and energy but pollutes the environment by producing green house gases also. In India, various leguminous crops are grown for

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pulses, cattle forage and green manuring. The major pulse (leguminous) crops include Chickpea (*Cicer arietinum*), Pigeon pea (*Cajanus cajan*), Black gram (*Vigna mungo*) and Green gram (*Vigna radiata*). India is the world's largest producer of pulses with its total pulse production contributing a quarter of world's total production. Black gram is one of the pulses, mostly produced in Asian countries due to the tropical climate and soil type. The annual production of black gram is 1.40 million tons grown over an area of 3.10 million hectares which produces a huge amount of lignocellulosic residue [2,3]. Black gram residue consists of cellulose (26.8 ± 2.3%), hemicellulose (32.48 ± 3.0%), lignin (23.14 ± 2.1%), crude protein (16 ± 0.8%), and ash content (5.1 ± 1.2%) [4].

Crop residues can serve as potential substrates for the production of various value added products like bioethanol, enzymes, organic acids, bio-surfactants, biogas, biohydrogen, and bio-fertilizers [5]. Crop residues are the inexpensive sources for the production of cellulases, hemicellulases and lignolytic enzymes. The hydrolysis of lignocellulosic biomass into fermentable sugars is the main factor for the high cost of ethanol production; the step of cellulase production accounts for 40% of total cost during ethanol production from cellulosic biomass [6]. Therefore, new microbial strains with higher production of cellulases and xylanases are required to reduce the cost of enzymes used in hydrolysis of lignocellulosic biomass [7]. Another strategy for cost reduction may be the use of low cost substrates for enzyme production and determination of optimum parameters of fermentation for enzyme production. Crop residues are very inexpensive materials for industrial production of enzymes. Solid state fermentation (SSF) can further reduce the cellulase and xylanase prices, because solid state fermentation results in more concentrated enzyme which is suitable for bioconversion of lignocellulosic biomass [8]. Cellulases and xylanases are very useful in many industries, such as textile, paper, bio-energy, animal feed, food and detergent. Cellulases and xylanases are predominantly used for enzymatic deinking and bio-bleaching in pulp and paper industry [9–12].

Biomass-degrading enzymes are produced by many microorganisms, like fungi, bacteria and actinomycetes. However, fungi are the major sources of cellulolytic and hemicellulolytic enzymes due to higher yield of enzyme production [12]. Among fungi *Trichoderma*, *Aspergilli*, *Penicillum*, are the main genera which are used for the production of cellulases and xylanases. *Trichoderma reesei* is the most efficient producer of cellulases but it is deficient in β-glucosidase activity, leads to build up of cellobiose which causes the end product inhibition during cellulase production and hydrolysis of lignocellulosic biomass. *Aspergillus* species are effective in production of high level of β-glucosidase enzyme [12–14].

The present study aims at optimising various cultural conditions for the maximum production of endoglucanase, FPase, β-glucosidase and xylanase from *Aspergillus nidulans* AKB 25 using various by-products of crops residues under SSF. The applications of crude enzyme were observed (a) to produce fermentable sugars from pearl millet residue by saccharification process and (b) to deink mixed office waste paper by bio-deinking process. According to the literature survey, this is the first report to produce crude enzymes from *A. nidulans* using black gram residue as the substrate under SSF.

2. Methods

2.1. Isolation and identification of microorganism

The fungal isolate AKB-25 was isolated from the soil samples collected from Jaipur, Rajasthan, India by using carboxymethyl cellulose containing media. The fungal isolate was maintained over potato dextrose agar slants at 4 °C. Molecular phylogenetic and morphological studies were carried out for identification of fungal strain AKB-25. Based on morphological characteristics and phylogenetic analysis of ITS1-5.8S-ITS2 gene sequences, fungal isolate AKB-25 was identified as *A. nidulans* and submitted to NFCCI, Agharkar Research Institute, Pune (India) with accession number NFCCI 2977. The ITS sequences of *A. nidulans* AKB-25 were submitted to GenBank with accession numbers KP734017.

2.2. Crop residues

Different crop residues such as sugarcane bagasse, black gram residue, corn stover, pearl millet stover, rice straw, rice husk, sugarcane tops, sun hemp residue, wheat bran, wheat straw were collected locally. The mature plants of black gram were harvested from the surface of soil without roots and seeds were separated from the plants. The remaining part except root and seeds was taken as 'black gram residue' for enzyme production. All the crop residues were washed, dried and chopped into 1–2 cm pieces with fodder cutter. Chopped pieces were ground into smaller particles in a Wiley mill. Particle size range 250–1400 μm was used for enzyme production under SSF without any pretreatment. Pearl millet stover was pretreated with 3% NaOH at 121 °C for 20 min and a solid to liquid ratio of 1:8 was maintained. Alkali pretreated pearl millet stover was repeatedly washed with tap water until neutral pH achieved and was used for hydrolysis studies without drying.

2.3. Enzyme production and extraction

Enzyme production was carried out under SSF using sugarcane bagasse, black gram residue, corn stover, pearl millet stover, rice straw, rice husk sugarcane tops, sun hemp residue, wheat bran, and wheat straw as carbon sources. 5 g of each of the substrates was moistened with Mandel Weber medium (77.5% initial moisture content) having the following composition as g/l: 1.4 $(NH_4)_2SO_4$, 2.0 KH_2PO_4, 0.3 $CaCl_2$, 0.3 $MgSO_4.7H_2O$, 0.1 Tween-80 and trace elements: 0.005 $FeSO_4{\cdot}7H_2O$, 0.0016 $MnSO_4{\cdot}7H_2O$, 0.0014 $ZnSO_4{\cdot}7H_2O$, 0.002 $CoCl_2{\cdot}6H_2O$. The initial pH of Mandel Weber medium for enzyme production was adjusted to 5.5. The pH of Mendel's mineral medium was adjusted with 1 N HCl or 1 N NaOH. 250 ml Erlenmeyer flasks containing 5 g of substrate were autoclaved at 121 °C for 30 min for sterilisation. Inoculum was prepared by harvesting spores from 7 days old wheat bran agar slants (3% wheat bran) by using 5 ml sterile distilled water containing 0.1% (w/v) Tween-80. Spore suspension was collected aseptically. Flasks were inoculated with 10^6 spores/gds and incubated at 30 °C for 6 days and left unperturbed. Initially, incubation time was kept as 6 days and after optimisation of incubation time flasks were incubated for 4 days. Enzyme was extracted by addition 50 ml of distilled