Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues

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ABSTRACT

Various agricultural and kitchen waste residues were assessed for their ability to support the production of a complete cellulase system by *Aspergillus niger* NS-2 in solid state fermentation. Untreated as well as acid and base-pretreated substrates including corn cobs, carrot peelings, composite, grass, leaves, orange peelings, pineapple peelings, potato peelings, rice husk, sugarcane bagasse, saw dust, wheat bran, wheat straw, simply moistened with water, were found to be well suited for the organism's growth, producing good amounts of cellulases after 96 h without the supplementation of additional nutritional sources. Yields of cellulases were higher in alkali treated substrates as compared to acid treated and untreated substrates except in wheat bran. Of all the substrates tested, wheat bran appeared to be the best suited substrate producing appreciable yields of CMCase, FPase and β-glucosidase at the levels of 310, 17 and 33 U/g dry substrate respectively. An evaluation of various environmental parameters demonstrated that appreciable levels of cellulases could be produced over a wide range of temperatures (20–50 °C) and pH levels (3.0–8.0) with a 1:1.5 to 1:1.75 substrate to moisture ratio.

1. Introduction

During the last few decades, interest in the use of lignocellulosic residues for biofuel production has increased due to their relative abundance, renewable nature and availability as almost zero cost substrates. Cellulose, the main component of lignocellulosic biomass, has attracted worldwide attention in its capacity to produce greener and cleaner fuels by producing fermentable sugars that can then be converted to second-generation bioethanol. The bioconversion of cellulose to fermentable sugars requires the synergistic action of complete cellulase system comprising of endoglucanases (EC 3.2.1.4) which act randomly on soluble and insoluble cellulose chains, exoglucanases (cellobiohydrolases; EC 3.2.1.91) which liberate cellobiose from the reducing and non-reducing ends of cellulose chains, and β-glucosidases (EC 3.2.1.21) which liberate glucose from cellobiose (Milala et al., 2005; Bansal et al., 2011; Deswal et al., 2011). The costs of cellulase account for more than 40% of the total processing cost (Ahamed and Vermette, 2008; Deswal et al., 2011). The availability of low-cost cellulases could be one solution to meet the increasing demand of biofuels. Hence, the use of low-cost technologies as well as cheaper substrates can help to reduce cellulase prices. Moreover, the ability of some

microorganisms to make use of lignocellulosic substrates as their growth medium to produce cellulases can make the bioconversions more economically viable.

Cellulases are produced by several microorganisms including bacteria, actinomycetes and fungi, but the latter are of great interest because they excrete their enzymes extracellularly (Bollok and Reczey, 2005). *Trichoderma reesei* is the most efficient producer of endo- and exo-glucanases (Miettinen-Oinnonen and Suominen, 2002), but does not excrete a sufficient amount of β-glucosidase (Bollok and Reczey, 2005) for which *Aspergillus* strains are known to be good producers (Juhasz et al., 2003). The major obstacle to using cellulosic residues for biofuel production is the recalcitrant nature, low yields and high cost of cellulases. The recalcitrant nature can be overcome by physical, chemical and thermal pretreatments while the enzyme yields can be enhanced by exploring the diverse environments for efficient natural microbial variants or tailoring the existing strains. On the other hand, the enzyme production costs can be reduced by adopting suitable fermentation processes that employ cheap and waste cellulosic residues as the inducers. Taking into consideration all the above mentioned problems, scientific dedication targets the economy of the cellulase production. Both solid and liquid fermentation systems have been used for enzyme production, but the former has greater advantages as it requires less capital, lower energy, a simple fermentation medium, has superior productivity, does not require a rigorous control of fermentation parameters and produces less wastewater.

Moreover, an easy control of bacterial contamination and lower costs of downstream processing make it more attractive (Pandey, 1994; Krishna, 2005; Sherief et al., 2010). Solid state fermentation (SSF) when carried out with different agricultural and kitchen waste residues adds value by decreasing the cost of enzyme production, reducing the quantity of solid waste and boosting the environmentally friendly management of agricultural and domestic wastes including corn cobs, carrot peelings, composite, grass, leaves, orange peelings, pineapple peelings, potato peelings, rice husk, sugarcane baggage, saw dust, wheat bran, wheat straw, which are increasing as a result of the rising population (da Silva et al., 2005; Bansal et al., 2011). The *Aspergillus* species are known to use a broad range of lignocellulosic substrates (Lockington et al., 2002; Kang et al., 2004; Wang et al., 2006; Gao et al., 2008) for the production of cellulases. In the present work, focus was placed on the production of complete cellulase complex from locally isolated *Aspergillus niger* under SSF on different agricultural and kitchen waste residues.

2. Materials and methods

2.1. Microorganism

A. niger NS-2 used in the present study was isolated locally from decaying agricultural residues (Bansal et al., 2011) and maintained on a potato dextrose agar.

2.2. Substrates and pretreatment

Various agro- and kitchen waste residues (corn cobs, carrot peelings, composite, grass, leaves, orange peelings, pineapple peelings, potato peelings, rice husk, sugarcane baggage, saw dust, wheat bran, wheat straw) were collected locally. These were dried by keeping them overnight in a hot air oven (70 °C), before being finely crushed and stored in air tight containers until further use. These substrates were pretreated separately with 1.0% (v/v) H_2SO_4 and 1% (w/v) NaOH. Twenty grams of each substrate was dispensed in 500 ml Erlenmeyer flasks, already containing 100 ml of acid or alkali and left at room temperature for 2 h. These were then washed thoroughly with distilled water to remove traces of acid and base followed by drying as mentioned above.

2.3. Cellulase production under solid state fermentation

Solid state fermentation was carried out in separate sets of 250 ml Erlenmeyer flasks, each having 5.0 g of untreated or treated (acid and alkali) dry substrates moistened with distilled water (pH 6.5) to obtain a final substrate to moisture ratio of 1: 1.5. These were autoclaved at 121 °C for 20 min, cooled and inoculated with 5 discs (7 mm) cut from the periphery of actively growing colonies of 72 h-old culture of *A. niger* NS-2 on PDA plates followed by incubation at 30 °C for 96 h under static conditions. The enzymes were extracted by adding 50 ml of tap water to the solid state cultures and shaking the contents on a rotary shaker at 150 rpm for 30–45 min at room temperature (Bansal et al., 2011). The contents of the flasks were then filtered through a metallic sieve and the solid residue was pressed to release leftover liquid, centrifuged (10,000g; 4 °C) for 10 min and the clear supernatant was analyzed for cellulase enzyme complex.

2.3.1. Enzyme assays

The components of the cellulase system were measured at 50 °C in terms of carboxymethyl cellulase (CMCase), filter paper activity (FPase) and β-glucosidase according to the methods of Mandels et al. (1976). One unit (U) of the CMCase, FPase, β-glucosidase was ex-

pressed as being equivalent to the enzyme that releases 1 μmole of glucose from CMC, Whatman filter paper and salicin respectively in 0.1 M acetate buffer, pH 4.0, in 1 min under assay conditions using dinitrosalicylic acid reagent (Miller, 1959). The enzyme productivities have been expressed in terms of U/g dry substrate (gds) used in the production medium.

2.4. Time course of cellulase production on wheat bran

Wheat bran was chosen as a substrate for further studies because no pretreatment was required for inducing the maximum production of enzyme components. The time course of enzyme production was studied by preparing different sets of 250 ml Erlenmeyer flasks, each containing 5 g wheat bran moistened with 1.5 parts of distilled water. These were sterilized and inoculated, as mentioned in Section 2.3, and incubated at 30 °C for 10 days. The flasks, in duplicate, were withdrawn at regular intervals of 24 h to study the production profiles of the cellulase system.

2.5. Optimization of environmental factors for cellulase production on wheat bran

The effect of various parameters such as incubation temperature (20–50 °C), substrate to moisture ratio (1:0.25–1:3.0), initial pH of the water used as a moistening agent (3.0–8.0), inoculum size (number of disc varied from 1 to 10) was investigated on the production profile of the cellulase system using wheat bran in SSF by varying one variable at a time.

3. Results and discussion

The use of abundantly available and cost-effective agricultural and kitchen waste residues that were once considered to be of no value are presently being recognized as raw materials of potential value (Karmakar and Ray, 2010) to achieve higher cellulase yields using SSF, thereby reducing the overall cost of enzyme production.

3.1. Evaluation of different agro- and kitchen waste residues for enzyme production in solid state fermentation

The production of cellulases with SSF is gaining interest as a cost effective technology with an almost tenfold predicted reduction in costs and much higher yields as compared to submerged fermentation (Tengerdy, 1996; Singhania et al., 2006). The nature of solid substrate is the most important factor in SSF for cellulase production as it not only supplies nutrients to the culture, but also serves as an anchorage for the microbial cells. Therefore, the particle size, chemical composition, cost and availability of the substrate are of critical importance during the selection of substrates. An ideal solid substrate should provide all the necessary nutrients to the growing microorganism for optimal function. However, some of the nutrients may be available in sub-optimal concentrations, or not even present in the substrate. In such cases, it would be necessary to supplement them externally. It has also been common practice to pretreat some substrates before use in SSF processes, making them more easily accessible for microbial growth (Pandey et al., 2001). The cellulase yields obtained on various raw and acid/alkali treated substrates are revealed in Tables 1–3. As compared to pre-treated substrates most of the untreated raw substrates had lower cellulase yields probably due to higher lignin content and firm binding, making them less accessible to the organism. Higher productivities of cellulases were noted in alkali-treated substrates than acid treated which can be related to the release of lignin component in the case of alkali treatment causing solubilization and modifications in the