

Full Length Research Paper

Cowpea waste: A novel substrate for solid state production of amylase by *Aspergillus oryzae*

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Cowpea waste moistened with minerals was used as the solid substrate for the production of glucoamylase by *Aspergillus oryzae* BS41. The waste supported mould growth and enzyme synthesis. Maximum glucoamylase synthesis (970U/ml) was obtained when fermentation was carried out at 72 h with an inoculum's size, initial moisture and pH of 4%, 60% and 5.0 respectively at $28 \pm 2^\circ\text{C}$. Supplementation with starch (2% w/v) as a carbon source enhanced glucoamylase production (1,500 U/ml) under optimized conditions while maltose adversely affected glucoamylase synthesis. Further supplementation with Soybean flour (3% w/v) gave a maximum amylase yield (2,333 U/ml) at 72 h. The study therefore presents cowpea waste as a cheap medium for the production of microbial amylase.

Key words: Cowpea waste, solid state fermentation, glucoamylase, *Aspergillus oryzae*.

INTRODUCTION

Solid state fermentation has been described as a process whereby insoluble substrate is fermented with sufficient moisture but without free water (Hesseltine, 1987). The system holds a tremendous potential for the production of microbial enzymes. It offers numerous advantages over submerged fermentation system including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipment etc (Nigam and Singh, 1994; Pandey et al., 2000).

Filamentous fungi have a number of properties which make them important both scientifically and industrially. They are widely cultured for the production of amylases on solid state fermentation process owing to their physiological, enzymological and biochemical properties such as their hyphal mode of growth and good tolerance to low water activity (A_w) (Raimbault, 1998).

The selection of a substrate for enzymes production in SSF depends on factors that are mainly related to cost and availability of the substrate and thus may involve screening of several heterogeneous products from agriculture or by-products of agro-industry. The substrate chosen must be the one that not only supplies the nutrients to the microbial culture growing on it but also

serve as an anchorage for the cells (Pandey, 2000). The substrate that provides the nutrients needed by the microorganism is considered as an ideal substrate. However, some of the nutrients may be available in sub-optimal concentrations or even absent in the substrates. In such case, it would become necessary to supplement them externally with other nutrients such as carbon and nitrogen (Pandey et al., 1995).

Some agro-industrial residues that have been used for the production of microbial amylases include rice bran (Akpan et al., 1999) wheat bran, (Pandey, 1993), copra waste (Pandey et al., 1995), tea waste (Selvakumar et al., 1998), cassava flour, oil palm waste, apple pomace, banana waste (Nigam and Singh, 1994; Pandey et al., 1994).

Cowpea waste is an agricultural by-products readily available and usually disposed off by the producers of bean cake and other products made from cowpea waste. This study reports the use of cowpea waste as substrate in solid state production of amylase.

METHODS

Micro-organism

Aspergillus oryzae BS41 was obtained from the Culture Collection Center of the University of Agriculture, Abeokuta. The culture was maintained on potato dextrose agar slants at 4°C and sub-cultured

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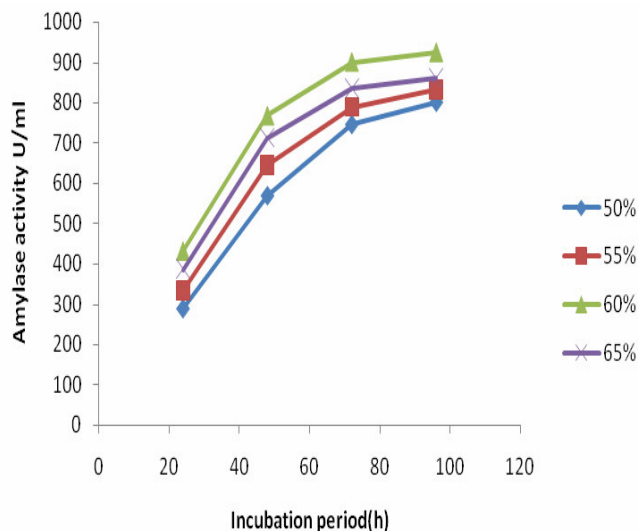


Figure 1. Effect of different initial substrate moisture on amylase yields by *A. oryzae* during SSF.

twice a month.

Pretreatment of substrate

Cowpea waste was collected from the University staff canteen. It was washed with excess distilled water and oven dried at 70°C for 12 h.

Solid state fermentation

A weighted quantity of cowpea waste (10 g) was moistened with an acidified mineral solution containing 3.5 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.8 mg $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 8.7 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 3.5 mg CaCl_2 per 100 g dry substrate. To study the effect of supplementation of carbon source, glucose, sucrose and starch were added at 1 - 5% w/v concentrations to the medium. Effect of nitrogen source was also carried out by addition of soybean flour, groundnut cake, and peptone at 1 - 5% w/v concentrations.

After adjusting the initial pH and moisture of the substrate to 4.8 and 60% respectively, it was autoclaved at 121°C for 15 min. The medium was inoculated with 4% v/w spore suspensions of *A. oryzae* BS41 and incubated at 30°C for 96 h.

Extraction of enzyme

Crude amylase extract was recovered by mixing a weighted quantity of fermented matter with 0.2 M acetate buffer (pH 4.8) in ratio 1:5 w/v. The mixture in a 250 ml conical flask was stirred on orbital shaker at 150 rpm for 2 h. The crude extract was filtered through glass fibre micro filter. The filtrate was assayed for amylase activity.

Enzyme assay

The amylase activity was assayed by measuring release of reducing sugars as described by Miller (1959). The reaction mixture

contained 4 ml of 5% (w/v) soluble starch prepared with 0.2 M acetate buffer (pH 4.8) and 1ml of crude amylase was incubated at 60°C for 1 h. Reducing sugars produced were determined by the dinitrosalicylic acid method. Glucoamylase activity was defined as the amount of enzyme that produced reducing sugar equivalent to one micromole of glucose from starch per minute at 60°C.

RESULT AND DISCUSSION

The experimental sample of Cowpea waste contained carbohydrate 56.61%, crude protein 3.92%, crude fibre 6.33% fat 10% and ash 3.14%. The initial moisture content was 20%.

Considering the contents derived through proximate analysis, it is a suitable substrate for that can be used for solid state bioprocessing. Solid state fermentations uses inexpensive agro-industrial materials as substrate and is distinguished from submerged fermentations by the fact that microbial growth and product formation occur at or near the surface of solid material of low moisture content (Lonsane et al., 1992). Moisture content and relative humidity have been reported to be the major factors in the bioprocessing of solid substrates. In this study four different moisture levels (50, 55, 60 and 65%) were tried for the production of glucoamylase by the cultures of *A. oryzae* BS41 on SSF. Maximum glucoamylase production (920 U/ml) was attained at 60% initial moisture content of the substrate after 72 h of cultivation (Figure 1). The result agrees with Pandey et al. (1995) that the substrate moisture content has a great influence on the physiological activity of the growing fungal culture. An increase in moisture content of the substrate to 64.5% was observed when fermentation was terminated at 96h. This might be due to the production of metabolic water by the fungi (Pandey et al., 1995).

The effect of inoculums size on glucoamylase production was determined by varying the number of spores added as inoculums (1, 2, 3, 4 and 5%) to the production medium. The maximum glucoamylase production of 980 U/ml was attained at 4% inoculum level at 72 h. A further increase in the inoculums size did not increase the amylase yield (Figure 2). The inoculum size has been reported as an important factor in SSF. A lower level of inoculum may not be sufficient for initiating growth and enzyme synthesis. An increase in inoculum size ensures a rapid proliferation of biomass and enzyme synthesis. After a certain limit, enzyme production could decrease because of depletion of nutrients due to the enhanced biomass, which would result in a decrease in metabolic activity (Kashyap, 2002). A balance between the proliferating biomass and available substrate materials would yield maximum enzyme (Pandey et al., 2000).

The effect of various carbon sources was evaluated at different concentration (Figure 3). A gradual increase in the production of glucoamylase over a period of 72 h was observed in the media supplemented with carbon sources. Among different carbon sources, starch appeared to have a significant effect on enzyme production followed by

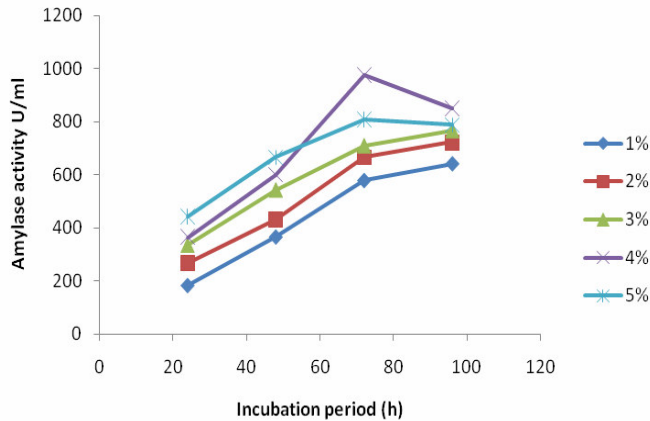


Figure 2. Effect of inoculum size on amylase yields by *A. oryzae*.

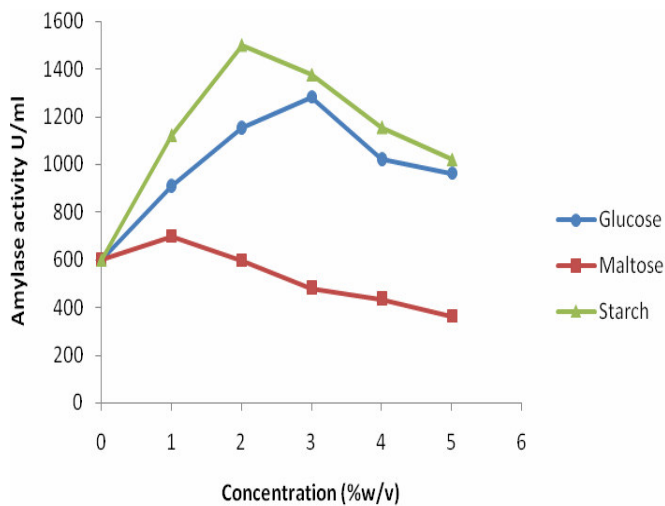


Figure 3. Effect of different carbon sources on amylase yields by *A. oryzae*.

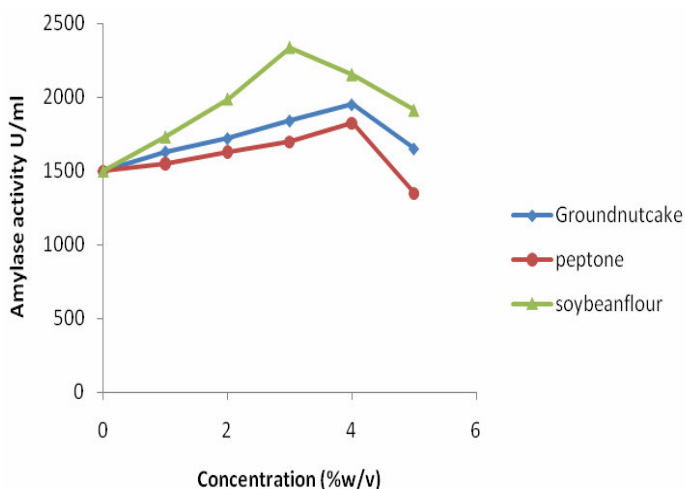


Figure 4. Effect of different nitrogen sources on amylase yields by *A. oryzae*.

glucose while addition of maltose adversely affected enzyme production by the fungi. The maximum enzyme yield observed in the medium with starch (2% w/v) was 1500 U/ml followed by glucose (3% w/v) with 1,264 U/ml while the unsupplemented cowpea waste gave 800 U/ml. An adequate supply of carbon as energy source is critical for optimum growth of organism and its metabolism (Akpan et al., 1999). The effect of various nitrogen sources on the production of glucoamylase is as shown in Figure 4. Among different nitrogen sources tested, Soybean flour was found to be the most suitable for enzyme production followed by groundnut cake and peptone. The maximum enzyme yield was 2,333 U/ml in the medium supplemented with soybean flour (3% w/v) while the enzyme yields obtained with groundnut cake (3% w/v) and peptone (4% w/v) at 72 h were 1,833 and 1,762 U/ml respectively. About three fold increase in amylase activity observed in the medium supplemented with soybean flour gave an indication that soybean flour contains high amount of protein. The result agrees with Akpan et al. (1999) that addition of soybean flour as organic nitrogen to rice bran medium enhanced amylase synthesis by *Aspergillus niger*. Supplementation of production medium by external nitrogen has been reported to be an important factor in SSF (Pandey, 1994). In this study increase in nitrogen in the medium resulted in an increase in an optimum C/N ratio which is important in any fermentation process and this resulted in an increase in the enzyme yields.

Based on the above results, it could be concluded that cowpea waste could be an attractive and promising substrate in solid state fermentation for the production of glucoamylase by *A. oryzae*. It could open new avenues for the bioconversion of this substrate into some value added products.

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