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Production of α-Amylase from *Penicillium chrysogenum* under Solid-State Fermentation by Using Some Agricultural By-Products

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Summary

Solid-state fermentation (SSF) was carried out using corncob leaf (CL), rye straw (RS), wheat straw (WS) and wheat bran (WB) as substrates for α-amylase production by a fungal culture of *Penicillium chrysogenum.* The effects of moisture level, particle size and inoculum concentration on enzyme synthesis from *P. chrysogenum* were investigated. Optimal moisture levels of substrates were 75, 65, 65 and 55 % for CL, WS, WB and RS substrates, respectively. Optimal particle size and inoculum concentration for the production of α-amylase were: >1 mm, 20 %; >1 mm, 20 %; 1 mm, 20 % and >1 mm, 30 % for CL, WS, WB and RS, respectively. WB showed the highest enzyme production with 160 U/mL under optimum conditions. The other enzyme activities were 28, 49 and 45 U/mL using CL, RS and WS, respectively.

Key words: α-amylase, *Penicillium chrysogenum*, solid-state fermentation

Introduction

The most widely used enzyme in the industry for starch hydrolysis is α-amylase (EC 3.2.1.1, α-1,4-glucan-4-glucanohydrolase), which catalyses the endocleavage of the α-1,4-glycoside linkages and the release of short oligosaccharides and α-limit dextrin. This enzyme is used commercially for the production of sugar syrups from starch which consist of glucose, maltose, and higher oligosaccharides (*1*). It is also extensively used in starch liquefaction and paper, food, pharmaceutical and sugar industries. To meet the demands of these industries, low cost medium is required for the fermentation of α-amylase (*2*).

Fungal α-amylases are produced by different fermentation techniques. Production of these α-amylases has been investigated through submerged (SmF) and solid-state fermentation (SSF) (*3*). The production of α-amylase by SmF using synthetic media has been studied by many researchers. The contents of synthetic media are very expensive and uneconomical, so they need to be replaced with more economically available agricultural by-products to reduce the costs. The use of SSF for enzyme production has many advantages over SmF due to its simple technique, low capital investment, lower levels of catabolite repression and better product recovery (*4*).

Bacteria, yeasts and fungi can grow on solid substrates and find applications in SSF processes. Filamentous fungi are the best adapted for SSF. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates (*5*).

The major factors that affect microbial synthesis of enzymes in a SSF system include selection of a suitable substrate and microorganism, particle size of the substrate, inoculum concentration and moisture level of the substrate. In SSF, the selection of a suitable solid substrate for the fermentation process is a critical factor and thus involves the screening of a number of agroindus-

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trial materials for microbial growth and product formation (*6*,*7*).

Wheat bran (WB) was obtained from a local flour mill. Corncob leaf (CL), rye straw (RS) and wheat straw (WS) were collected from fields. These substrates are used as cattle feed.

The present work represents an investigation into α-amylase production by SSF with WB, CL, RS, and WS as substrates and the determination of optimized production conditions.

Materials and Methods

Fungus

Penicillium chrysogenum used in this study was isolated from the air in Edirne city and identified by Asan *et al.* (*8*). It was found to be a good α-amylase producer and its enzyme property was investigated in our previous study (*9*). It was maintained on potato dextrose agar slants at 4 °C.

Inoculum preparation

A volume of 7 mL of sterile distilled water was transferred to a sporulated (7-day-old) PDA slant culture. The spores were dislodged using the inoculation needle under aseptic conditions and the suspension, with appropriate dilution, was used as inoculum. A volume of 1 mL of spore suspension contained about $5 \cdot 10^6$ spores.

Solid-state fermentation

Masses of 5 g of dry substrates were taken into 250-mL Erlenmeyer flasks. To adjust moisture levels (% by mass per volume), 0.1 M acetate buffer (pH=5.0) was added. The contents of the flasks were mixed thoroughly and autoclaved at 121 °C for 20 min. The flasks were incubated at 28 °C for 7 days.

Optimization of process parameters

Various process parameters affecting enzyme production during SSF were optimized. The strategy was to optimize each parameter independently of the others and subsequently optimal conditions were employed in all experiments.

In a sequential order, the various process parameters were optimized for maximal enzyme production by using different solid substrates such as WB, CL, RS and WS. The tested process parameters were initial moisture content (45, 55, 65 and 75 %, by mass per volume), particle size (>1, 1, 0.85 and 0.60 mm) and inoculum concentration (10, 20, 30, 40 and 50 %, by volume per mass).

Determination of dry mass of substrates

Dry mass of substrates was determined by drying them in an oven at 80 °C for 24 h.

Enzyme extraction

Fermented substrates were mixed thoroughly with 0.1 M acetate buffer (pH=5.0, 50 mL) containing 0.1 % Tween 80 (*3*). Contents were mixed by shaking for one hour at 30 °C on a rotary shaker at 220 rpm. The slurry was squeezed through muslin cloth. The extract was filtered through a Whatman No. 1 filter paper and the filtrate was used as the crude enzyme.

Enzyme assay

Soluble starch (0.8 %) was dissolved in boiling 0.1 M acetate buffer (pH=5.0) and then cooled to 30 °C. Fresh iodine reagent was prepared by diluting 1.0 mL of stock solution (0.5 % I_2 in 5.0 % KI) in 500 mL of distilled water containing 5 mL of 5 M HCl. For the assay, 0.1 mL of enzyme solution and 0.2 mL of soluble starch solution were incubated at 30 °C for 5 min. The reaction was stopped by adding 5 mL of iodine reagent. The absorbance was measured at 620 nm against a blank (*10*). One unit of α-amylase is defined as the amount of enzyme which hydrolyses 0.1 mg of starch in a minute at 30 °C when 1.6 mg of starch is present.

Results and Discussion

Fig. 1 shows the effect of moisture level on enzyme production for CL, RS, WS and WB substrates. Optimal moisture levels of substrates were found to be 75, 55, 65 and 65 % for CL, RS, WS and WB, respectively. Enzyme productions were 20, 34, 40 and 127 U/mL with CL, RS, WS and WB, respectively. Moisture content is a critical factor for SSF processes because this variable has influence on growth and biosynthesis and secretion of different metabolites. Lower moisture content causes reduction in solubility of the nutrients of the substrate, low degree of swelling and high water tension. On the other hand, higher moisture levels can cause a reduction in enzyme yield due to steric hindrance of the growth of strain by reduction in porosity (interparticle spaces) of the solid substrate, thus interfering with oxygen transfer (*11*). The moisture levels in SSF processes vary between 30 and 85 %. The optimum moisture content for growth and substrate utilization depends on the organism and the substrate used for cultivation. Cultivation of *Aspergillus niger* on starchy substrates such as cassava and wheat bran requires considerably lower moisture levels than on coffee pulp or sugarcane bagasse. This is proba-

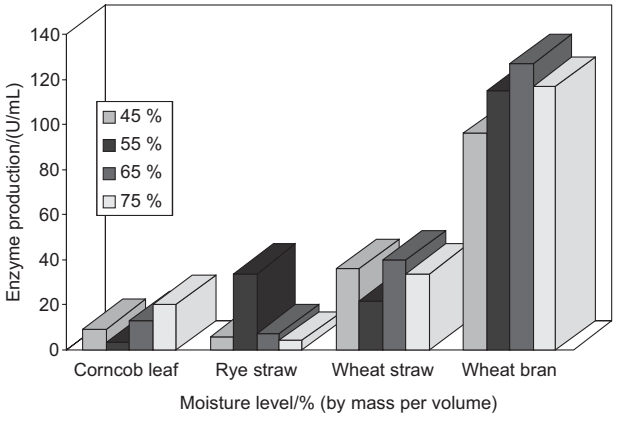

Fig. 1. Effect of initial moisture level (% by mass per volume) on α-amylase production in an SSF system. Inoculum fraction was 20 % (by volume per mass). Process conditions: temperature 28 °C, incubation time 7 days

bly because of the greater water holding capacity of the former substrates (*5*).

As shown in Fig. 2, appropriate inoculum concentrations were 20 % for CL, WS and WB and 30 % for RS. Enzyme productions were 25, 40, 45 and 152 U/mL with CL, RS, WS and WB, respectively. Inoculum level was an important factor for the production of α-amylase. Higher inoculum concentration increased the moisture content to a significant extent. The free excess liquid present in an unabsorbed form will therefore give rise to an additional diffusional barrier together with that imposed by the solid nature of the substrate and lead to a decrease in growth and enzyme production (*4*). Lower inoculum level results in a lower number of cells in the production medium. This requires a longer time to grow to an optimum number to utilize the substrate and form the desired product (*12*).

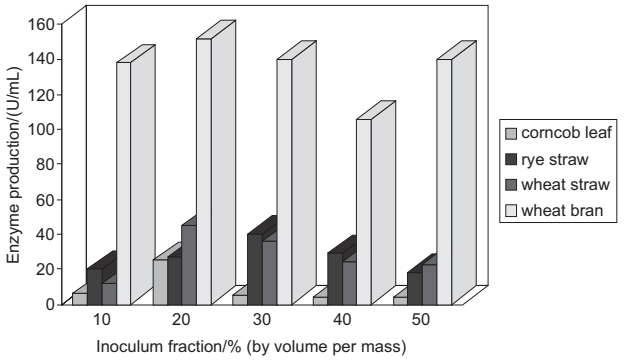

Fig. 2. Effect of inoculum fraction (% by volume per mass) on α-amylase production in an SSF system. Process conditions: initial moisture levels 75, 55, 65 and 65 % for CL, RS, WS and WB substrates, respectively, temperature 28 °C, incubation time 7 days

The particle size (specific surface area) is also very important for SSF. Fig. 3 shows the effect of substrate particle size (mm) on α-amylase production in an SSF. Enzyme productions were 28, 45, 49 and 160 U/mL with CL, RS, WS and WB substrates, respectively. Particle sizes of >1 and 1 mm favoured α-amylase production. Larger particles provide better respiration/aeration efficiency (due to increased interparticle space). In contrast, a small substrate particle may result in substrate accumulation, which may interfere with microbial respiration/aeration and therefore result in poor growth and enzyme production (*13*). The lower enzyme productions were obtained at small particle sizes (0.6 and 0.85 mm). Krishna and Chandrasekaran (*14*) reported that banana fruit stalk particles of 400 μm favoured maximal α-amylase production compared to larger particles. On the other hand, Baysal *et al*. (*4*) reported that WB and RH with particle size of 1000 and 500 μm, respectively, favoured α-amylase production.

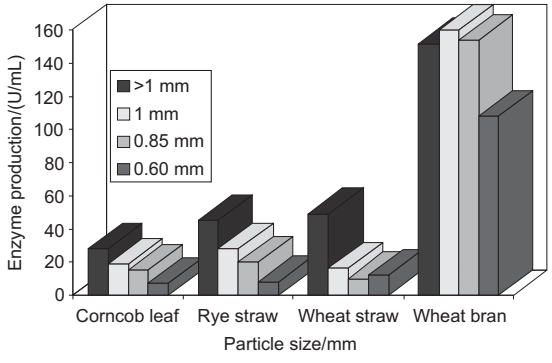

Fig. 3. Effect of substrate particle size (mm) on α-amylase production in an SSF system. Process conditions: initial moisture levels 75, 20, 55 and 30 % for CL, RS, WS and WB, respectively, inoculum fraction 65, 20, 65 and 20 % for CL, RS, WS and WB, respectively, temperature 28 °C, incubation time 7 days

Conclusions

The use of SSF for production of α-amylase using *P. chrysogenum* is an economical process and is very simple to apply. All the substrates supported biosynthesis of α-amylase using *P. chrysogenum* under SSF. CL, WS, RS can be used as substrates for enzyme synthesis in SSF. However, these substrates did not cause enzyme productions as high as WB. Therefore, WB was superior to other substrates for synthesis of α-amylase from *P. chrysogenum* by SSF.

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