Alpha Amylase Production by Aspergillus Oryzae Using Solid State Fermentation

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Abstract

A fungal strain of Aspergillus oryzae was used for the production of alpha amylase by solid state fermentation from agro-industrial waste. Enzyme production was growth associated and maximum activity (8.23 U/ml) were obtained after 120h when incubated at 30°C on wheat bran with initial moisture content 60%; initial medium pH = 5 . Enzyme activity increased when the solid medium was supplemented with additional nitrogen source (sodium Nitrate).

Keywords: Alpha amylase, Aspergillus oryzae , solid state fermentation.

إنتاج إنزيم ألفا-أميليز بوساطة Aspergillus Oryzae باستخدام التخمرات الصلبة

الخلاصة

تم في هذه الدراسة استخدام الفطر *Aspergillus oryzae* لإنتاج إنزيم ألفا-أميليز من المخلفات الصناعية بطريقة تخمرات الحالة الصلبة (SSF) وقد تم الحصول على أعلى قيمة لإنتاج الإنزيم (8.23U/ml) عند استخدام نخالة الحنطة كمادة أساس بنسبة ترطيب 60% وأس هيدروجيني أولي 5 بعد فترة حضانة 120ساعة في درجة حرارة 30°م وقد لوحظ أن إنتاجية الإنزيم قد زادت عند إضافة المصدر النايتروجيني (نترات الصوديوم) إلى وسط التخمر.

Introduction

Amylase are well known starch degrading enzymes, which are comprised into α-, β-, and γ-amylase. Among these α-amylase (endo-1,4, α-D-glucan glucohydrolase, EC.(3.2.1.1) is an extracellular enzyme that randomly cleaves the 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylose chain (1). Fungal and bacterial amylases are widely used for the commercial applications in food processing industries (2) Fungal amylases particularly from *Aspergillus* species, find various applications in antistalling (baking industry), haze clarification in fruit juices and alcoholic beverages, glucose and maltose syrup production and other food products(3) these amylases have a high efficiency in saccharification of starch when compared to bacterial α-amylases(4). *A. oryzae* has an efficient system for secretion of proteins and is extensively used to produce industrial enzymes (5). Solid-State Fermentation (SSF) is widely established for the production of enzymes by filamentous fungi(6,7). SSF utilizes various agro-industrial wastes as substrate that act

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as physical support and source of nutrients (8,9) food and

agricultural wastes can serve as substrates for the

Production of various fermented products andenzymes (10). SSF offers advantages such as high volumetric productivity, better product recover and product characteristics, low capital investment, reduced levels of catabolite repression, value addition of agricultural industrial wastes reducing

pollution problems and less effluent generation (11).

This study deal with a variety of easily available and inexpensive agro-industrial substrates for the production of α-amylase using *Aspergillus oryzae* using SSF.

Materials and Method

Microorganisms

A fungal strain of *Aspergillus oryzae* procured from biotechnology division. Potato dextrose agar (PDA) was used for growing and maintaining the culture. The fully sporulated slants were obtained after 7 days of incubation of newly streaked slants at 30°C and were stored at 4°C and sub-cultured fortnightly.

Substrates:

Substrates obtained from local markets were, corn waste, bagass, rice bran, wheat bran and potato residue.

Preparation of inoculation

To the 7 days old culture slants, 10ml of 0.1% Tween-80 solution was added and the spores were dislodged using an inoculation needle under sterial conditions. Spores in the solution were collected in asterial flask and the suspension was diluted appropriately for the required spore density. The spores count was

made on a haemocytometer slide bride(12).

Solid State Fermentation

Dry substrate (10g) was taken into an erlenmeyer flask (250ml) added with 5ml of mineral solution

containing (KH_2PO_4 2, NaCL 1; $MgSO_4 7H_2O$ 1g/L distilled water) to obtain an initial moisture level of 50% content of flasks were mixed and autoclaved at 121°C for 20 min. after cooling at room temperature spore suspension (1ml , density, 1×10^5 spores/ml) was used as the inoculums. Inoculated flasks were incubated at 25°C for 120h.

Enzyme Extraction

The fermented solid mass was mixed with 50ml distilled water containing 0.1% Tween-80 and agitated on a rotary shaker for 30min. whole contents were filtered with whatman No.1 and centrifuged at 10000rpm for 10min and the clear

supernatant was used as the crude enzyme source.

Enzyme Assay

Alpha amylase activity was determined (13). Reaction mixture contained: 1% soluble starch, 1.25ml; 0.1M acetate buffer (pH=5), 0.5ml; crude enzyme extract, 0.25ml. After 10min of incubation at 50°C, liberated reducing sugars (glucose equivalents) were estimated by 3,5-dinitrosalicylic acid (DNS) method of miller(14). The color developed was read at 510nm using APELPD-

303 spectrophotometer. Glucose was used as the standard.

Blank contained: 1% starch solution, 1.25ml; 0.1M acetate buffer (pH = 5), 0.5ml; DNS and crud enzyme 0.25ml.

One unit (IU) of α-amylase is defined as the amount of enzyme releasing one µmol glucose equivalent per minute under the assay conditions.

Optimization of cultural conditions

Various physical and chemical parameters such as carbon sources (corn waste, bagasse, wheat bran, rice bran and potato residue), initial moisture content (50,55,60,65 and 70%, effect of inorganic nitrogen (0.25M) ammonium chloride, ammonium sulphate and sodium nitrate) and organic nitrogen (1%w/w) sources (pepton and yeast

extract) and effects of temperature (20,25,30,35,40 and 45°C) and pH (3,4,5,6,7 and 8) were studied. All the experiments were conducted in triplicate and values were averaged.

Results and discussion

Determination of the optimum carbon source

Among 5 substrates screened (Fig.1), wheat bran (WB) gave highest enzyme production (4.31U/ml) which was almost 2 times higher than that produced by other substrates. WB has been a highly reported substrate producing promising results, among the various agro-industrial substrates used (15,16,17) widespread suitability of WB may be due to the presence of sufficient nutrients and its ability to remain loose even in moist conditions, thus providing a large surface area.(18).

Effect of moisture on α-amylase production

Fig.2 showed that alpha amylase production increased with an increase in initial moisture content with a maximum at level 60%. In most of the cases, 40-70% moisture requirements have been reported for maximum growth and substrate utilization.(19).

Although the fungal growth occurred at a lower moisture

level (45%), it was associated with early sporulation and a

significant reduction in the enzyme yield. This could be due to the non-availability of nutrients as lower moisture content has been known to reduce solubility of nutrients of the substrate, a lower degree of swilling and high water tension affecting microbial activity. Drastic reduction in enzyme activity occurred at initial moisture content 70%. This was because high moisture level decreased porosity of particles, developed stickiness of substrate resulting in agglomeration, and reduced gas volume and gaseous diffusion resulting in low oxygen transfer.(20)

Effect of nitrogen sources on α-amylase production

In the present work, different organic nitrogen sources such as pepton, yeast extract non of the supplied organic nitrogen sources (pepton, yeast extract) showed any positive effect on the enzyme production although they promoted good fungal growth (Fig.3) . Inorganic nitrogen additives (ammonium chloride, ammonium sulphate) also exerted negative effect on the microbial activity and resulted in lower enzyme activity.(21) have reported the insignificant effect of ammonium sulphate and ammonium chloride on α-amylase production by *A. oryzae*. While supplementation of sodium nitrate increased enzyme yields marginally since this supplement gave similar enzyme activity, sodium nitrate (0.25M) which gave higher specific activity was selected for further optimization studies.

Effect of pH on α-amylase activity

Each organism processes a characteristic pH range for its growth and activity with an optimum value in between the range(22) The pH of culture mainly changes due to the microbial metabolic activities(23). Enzyme synthesis occurred at the pH range 3-8 (Fig.4) and optimal enzyme

activity (7.15 U/ml) were obtained at an initial pH of 5.

Effect of Temperature on α-amylase activity

Enzyme synthesis occurred between 20-45°C with an optimum at 30°C (Fig.5). A decrease in enzyme activity was observed when temperature rang fell outside the mesophilic range. Similar results have also been previously reported for *A. oryzae* by (21) and (23).

Conclusions

In accordance with the results, taking all the influencing factors and the results into consideration, the optimal cultural process was considered as follows: media and cultural conditions including wheat bran waste with initial moisture content 60%; initial medium pH = 5 , culture temperature 30 °C and culture time 120 hours . The results indicate the suitability of using cheap and abundantly available agro-industrial waste as solid substrate for large-scale production of amylase in an SSF system in order to reduce the high costs. Maximum utilization of this waste can also contribute to efficient solid-waste management, where continuous accumulation of industrial wastes poses a serious environmental problem.

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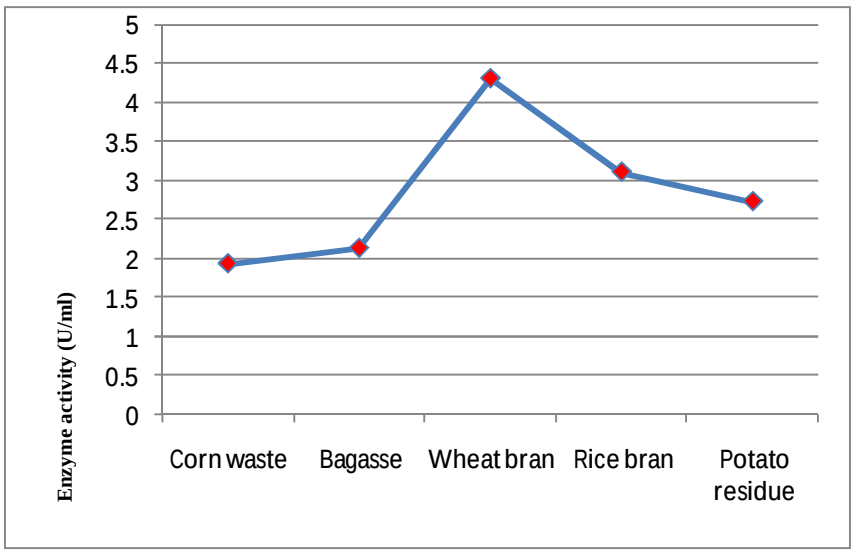

Figure (1) Determination the optimum carbon source of α-amylase production by Aspergillus oryzae using SSF

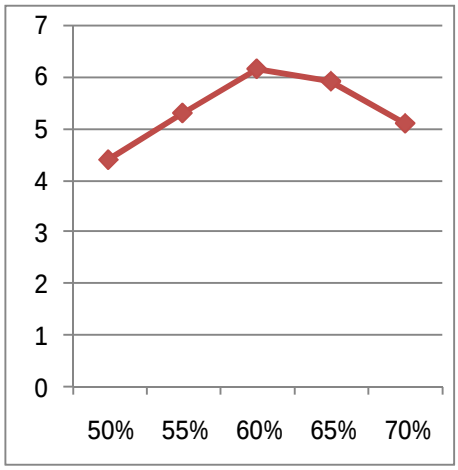

Figure 2- Effect of moisture% on α-amylase production by *Aspergillus oryzae* using SSF

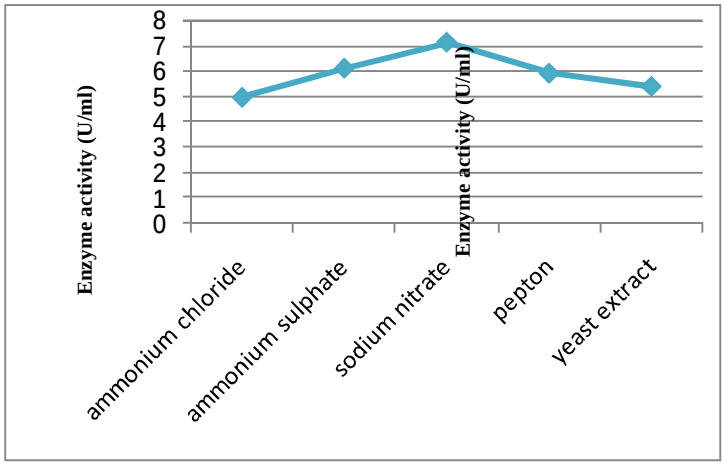

Figure3- Effect of nitrogen source on α-amylase production by Aspergillus oryzae using SSF

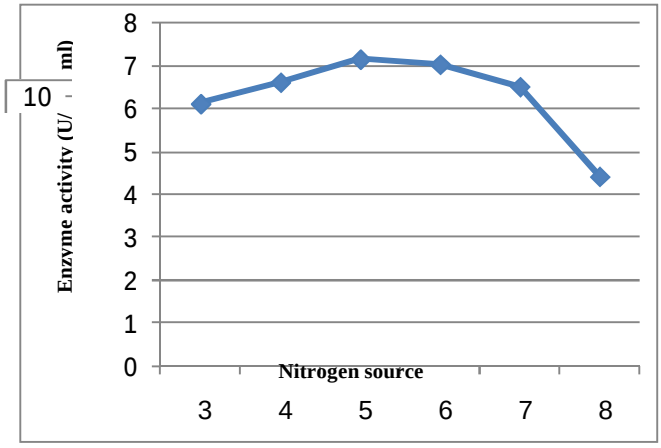

Figure4- Effect of pH on α-amylase production by*Aspergillus oryzae* using SSF

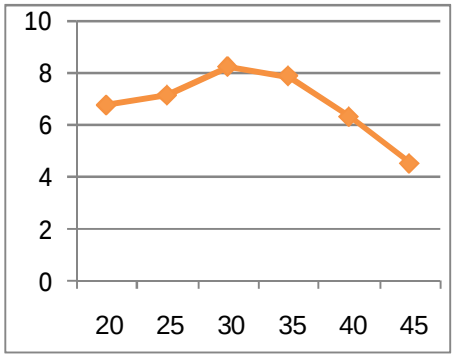

Figure 5- Effect of Temperature on α- amylase production by Aspergillus oryzae using SSF