



Original Research Article

Production and optimization of Glucoamylase from *Aspergillus oryzae* NCIM 1212 using wheat bran, varying chemical parameters under solid state fermentation

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ABSTRACT

Keywords

Glucoamylase, *Aspergillus* species, Solid state fermentation, chemical parameters

Glucoamylase is a well recognized amylolytic enzyme used in food industry, which is generally produced by, *Aspergillus niger*, *Aspergillus wentii*, *Aspergillus terreus* and *Aspergillus oryzae*. Its production was optimized under solid state fermentation in potato dextrose agar medium. Different substrates like wheat bran, rice bran and coconut oil cake which were purchased from the local market are checked for optimum production. During screening it was found that wheat bran using *Aspergillus oryzae* was more productive than any other source. Optimized production was obtained by varying the chemical parameters such as carbon supplements, Organic and inorganic nitrogen supplements. The following observations were made: Fructose as a carbon supplement gave activity 42.32 U/ml. Peptone acted as a good nitrogen supplement resulting in activity of 45.21 U/ml. Ammonium sulphates as a good inorganic nitrogen supplement, gave an activity of 32.25 U/ml.

Introduction

Glucoamylase (E.C. 3.2.1.3) is an enzyme that breaks the glucose units from the non reducing sides of amylose chain, glycogen and amylopectin involving in the hydrolysis of α (1-4) faster than α (1-6), α (1-3) linkages and produce producing D glucose in successive manner (Joshi VK, Pandey A et al (1999) Vasudeo Zambare 2010). The amylase family has two major classes, namely amylase (EC 3.2.1.1) and

glucoamylase (EC 3.2.1.3). α - Amylase can hydrolyze starch into maltose, glucose and maltotriose by cleaving the 1, 4-a-D-glucosidic linkages between adjacent glucose units in the linear amylose chain. Glucoamylase hydrolyses single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner, and produce glucose as the sole end-product from starch and related

polymers. Unlike α -amylase, most glucoamylases are also able to hydrolyze the 1, 6-glycosidic linkages at the branching points of amylopectin, although at a lower rate than 1, 4-glycosidic linkages (Fogarty, W.M, (1983), Antranikian.G, (1992), RiteshParbat et al (2011).

Glucoamylases are industrially important hydrolytic enzymes of biotechnological significance and are currently used in food and pharmaceutical industries. (Joshi VK, Pandey A et al (1999), Vasudeo Zambare (2010), James JA and Lee BH (1992).

Glucoamylases mainly used in the production of glucose syrup, high fructose corn syrup, and alcohol (James JA and Lee BH, 1992). Glucoamylases are produced by various microorganisms, including bacteria; fungi and yeasts, but almost exclusively in filamentous fungi and far less in bacteria and yeast (RiteshParbat et al 2011). These enzymes have found wide applications in the processed food industry, fermentation industry, and textile and paper industries (Selvakumar, et al (1999) Pandey A et al (1999) Tengerdy RP, 1998)

Traditionally, glucoamylase has been produced by SmF and used in a one-way process in solution. In recent years, however, the solid state fermentation (SSF) processes have been increasingly applied for the production of this enzyme. SSF holds tremendous potential for the production of enzymes (Pandey A et al 1999). It can be of special interest in those processes where the crude fermented product may be used directly as enzyme source (Tengerdy RP, 1998).

The exclusive production of glucoamylases is achieved by *Aspergillus niger* (Wang X J et al (2006) Shruti Puri et

al (2013). and *Aspergillus oryzae* in enzyme industry (Shruti Puri et al (2013) Biesbeke R et al (2005). Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Spectrum of application of amylases has widened in many sectors such as clinical, medicinal and analytical chemistry. Current developments in bio-technology are yielding new applications for enzymes. In the last decades, there has been an increasing trend towards the utilization of the solid state fermentation (SSF) to produce several enzymes from microorganisms (Shruti Puri et al (2013) Sodhi H K et al 2005). The food, beverage and agro industries produce large quantities of residues that pose serious problems of disposal, in spite of them being sources of biomass and nutrients. These substrates are used for production of valuable compounds such as enzymes and various secondary metabolites (Shruti Puri et al (2013) Socol C R et al (2003). Agro-industrial residues are generally considered the best substrates for SSF processes and use of SSF for the production of enzymes is no exception to that (Shruti Puri et al (2013) Ellaiyah P et al (2002). Use of suitable low cost fermentation medium for production of alpha amylase using agricultural by-products has been reported (Haq et al 2003). Wheat bran, paddy husk, rice processing waste and other starch containing wastes have gained importance as supports for growth during enzyme production (Anto et al 2006).

Sources and forms of gluco amylases:

Glucoamylase's may be derived from a wide variety of plants, animals and microorganisms, though most glucoamylase's occur in fungi. The enzymes used commercially originate

from strains of either *Aspergillus Niger* or *Rhizopus sp.* where they are used for the conversion of malto-oligosaccharides into glucose (Fogarty et al (1983) and Pandey et al 1995). These enzymes are generally regarded as safe (GRAS) by the Food and Drug Administration (FDA). The properties of glucoamylase have been reviewed comprehensively elsewhere. Since the discovery of two forms of glucoamylase from black koji mold in the 1950's, many reports have appeared on the multiplicity of glucoamylase. The various forms of glucoamylases are thought to be the result of several mechanisms: mRNA modifications, limited proteolysis, variation in carbohydrate content or the presence of several structural genes (Pretorius et al 1991).

Substrates: Commonly used carbon sources are dextrin; fructose, glucose, lactose; maltose and starch are very expensive for commercial production of these enzymes. various agricultural by products like wheat bran (WB),groundnut oil cake (GOC), rice husk (RH),coconut oil cake (COC) sugarcane bagasee (SB) potato residue (PR) rice brain (RB),green gram bran (GGB),black gram bran (BGG) and maize bran (MB)etc are abuntely used

Materials and Methods

Microorganisms and Maintenance

The organisms used in the present study were procrued from the National Collection of Industrial Microorganisms (NCIM), Pune and The Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The cultures were maintained on potato dextrose agar slants. Sporulation medium was poured into culture test tubes and autoclaved for 15 minutes at 121°C temperature and 20 psi

pressure for sterilization. Then tubes were laid in the slanting position for the solidification of the medium (Rashid et al 1997). The slants were inoculated with spores of desire organisms and finally placed in incubator at 25 - 30°C for 5-6 days. Fully matured slants were stored at 4°C and renewed once a month.

1. *Aspergillus niger* NCIM 616
2. *Aspergillus oryzae* NCIM 1212
3. *Aspergillus terreus* MTCC 1782
4. *Aspergillus wentii* MTCC 1901

Maintenance of culture

The cultures were maintained on PDA slants and incubated for 5 days at 28 °C. The organisms were sub cultured every month.

Inoculum preparation

A piece of culture from 5 days old slant was used to inoculate in seed flask containing 5g substrate with 100% moisture and incubated for 5 days at 30°C. After incubation, fermented dough was mixed aseptically followed by addition of 50 ml of saline containing 0.1% Tween-80. After 20 min the mixture was filtered off through sterile glass wool to get spores. Spore count was determined by serial dilution and spread plating method

Enzyme production in Solid state fermentation (SSF)

The static experiments were conducted in 250 ml Erlenmeyer flasks containing 5 g of substrates and then moistened with 5ml of water and sterilized at 121 °C for 30 min. The fermentation process was started by adding 1 ml of spore suspension (5×10^7 spores/ml) as prepared above. The whole content was mixed thoroughly and then incubated at 30°C for 5 days.

Screening of substrates

The substrates used in this study namely groundnut oil cake, rice bran, wheat bran and coconut oil cake were purchased from local market in Visakhapatnam. These substrates were dried in an oven at 105°C to a constant weight and then used to study their effect on the glucoamylase production. The best solid substrate achieved by this step was fixed for subsequent experiments.

Optimization process

Fermentation process is governed by a large number of chemical factors. The protocol adopted for the optimization of process parameters was to evaluate the effect of an individual parameter at a time and to incorporate it at the standard level.

Effect of chemical parameters

Effect of carbon source

Influence of various carbon supplements on enzyme production was carried out by adding various carbon supplements namely glucose, fructose, maltose, starch and sucrose a 1% (w/w) to fermentation medium.

Effect of organic and nitrogen source

Influence of various organic nitrogen supplements on enzyme production was studied by adding various supplements namely urea, soya bean meal, peptone, yeast extract, malt extract sodium nitrate, ammonium sulphate, ammonium chloride and potassium nitrate at 1% (w/w) to fermentation medium

Extraction of crude enzyme

To the fermented dough 50 mM citrate

buffer (pH 5) (1:10) was added and homogenized for 2 hours with a constant stirring at room temperature. This suspension was filtered through Whatman filter paper number 1 and the filtrate was again centrifuged at 6000 rpm for 15 min. This solid-free supernatant was used as enzyme source for assaying glucoamylase activity.

Assay For Glucoamylase

(Ritesh Parba and Barkha Singhal, 2011)

Glucoamylase activity was determined by taking an appropriate amount of the enzyme followed by the addition of 1 % soluble starch solution in 50 mM citrate buffer (pH=5.5) at 50°C for 20 min. The released glucose was measured with 3, 5-dinitrosalicylic acid (DNS) reagent using glucose as a standard (Miller, 1959). Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing one μ mole of glucose equivalent per minute per ml.

One unit of glucoamylase was defined as the amount of enzyme required to release 1 μ mol of glucose/min under the assay conditions and activity was expressed as U/mL. The activity was also expressed as U/mg biomass, and U/mg proteins. Similar assays for glucoamylase have been used by other researchers (Mahajan et al. 1983)

Standard curve (DNS Method for Glucose)

Dinitro salicylate reagent

Dissolve 1.6gm of NaOH in 20ml of distilled water, then dissolve 1g of 3,5-Dinitro salicylate acid in above NaOH solution. Dissolve 20gm of sodium potassium tartarate in 50ml of H₂O in a separate beaker and mix with dinitro

salicylic acid solution and finally make up to 100ml with distilled water.

Standard solution of glucose (1mg/ml)

Pipette out different volumes of glucose (0.2-1ml) into different test tubes and make the volume to 1ml with water. Add 1ml of dinitrosalicylate acid in each test tube and keep in a boiling water bath for 10min. Cool and add 10ml of distilled water in each test tube and measure the orange red colour developed at 520nm in a photoelectric calorimeter.

Calculation

Unit (U/ml) = (mg of glucose liberated)(1000)/(molecular weight of glucose)(Volume of supernatant used in calorimetric determination)
Volume of supernatant used in calorimetric determination=1ml

Results and discussion

In the present study agro-residues and microorganisms were screened for their potential to serve as substrate and fungal supplements for glucoamylase production in SSF. The process parameters for the production of glucoamylase by *Aspergillus oryzae* NCIM 1212 strain grown on wheat bran substrate were done under SSF. For enzyme production process of chemical parameters like the basal medium was supplemented with different nutrients in the form of carbon supplements, organic and inorganic nitrogen supplements. The results are presented and discussed in view of existing literature.

Chemical parameters

Effect of carbon supplements on glucoamylase production

Influence of various carbon supplements

on enzyme production was studied by adding various supplements namely glucose, fructose, maltose, starch and sucrose a 1% (w/w) to fermentation media. Of the five different carbon supplements used as enrichment, fructose was a good carbon supplements which gave maximum glucoamylase activity 42.32 U/ml (Figure 2). Fructose was the best carbon supplements, as it provides the much required carbon in substrate.

Zambare et al (2010) reported maximum production of amylase when fructose was the carbon supplement.

Effect of organic nitrogen supplements on glucoamylase production

Influence of various organic nitrogen supplements on enzyme production was studied by adding various supplements namely urea, soya bean meal, peptone, yeast extract and malt extract at 1% (w/w) to fermentation media. Of the five different organic nitrogen supplements used as enrichment, peptone was a good nitrogen supplements which gave maximum glucoamylase production 45.21U/ml.(figure 3). Peptone was the best organic nitrogen supplements as it provides the much required nitrogen in substrate.

Imran et al (2011) reported that, peptone enhanced the glucoamylase production

Effect of inorganic nitrogen supplements on glucoamylase production

Influence of various inorganic nitrogen supplements on enzyme production was studied by adding various supplements namely sodium nitrate, ammonium sulphate, ammonium chloride, potassium nitrate at 1% (w/w) to fermentation media.

Fig.1 Glucose Standard Curve

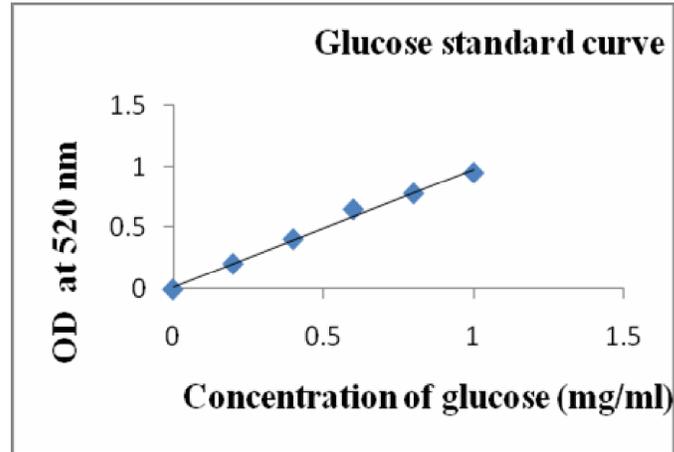


Fig.2 Effect of carbon supplements on glucoamylase

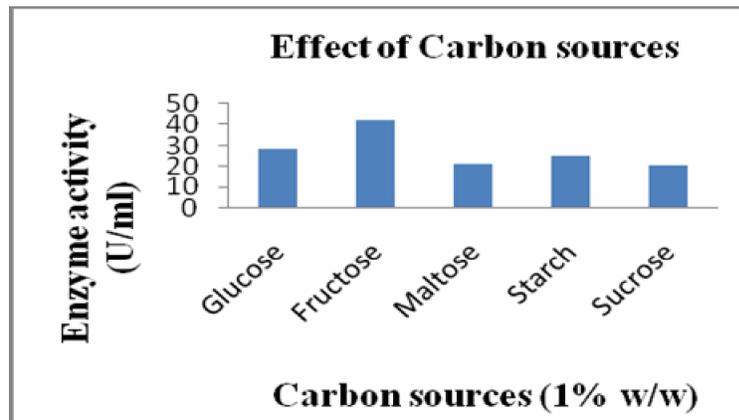
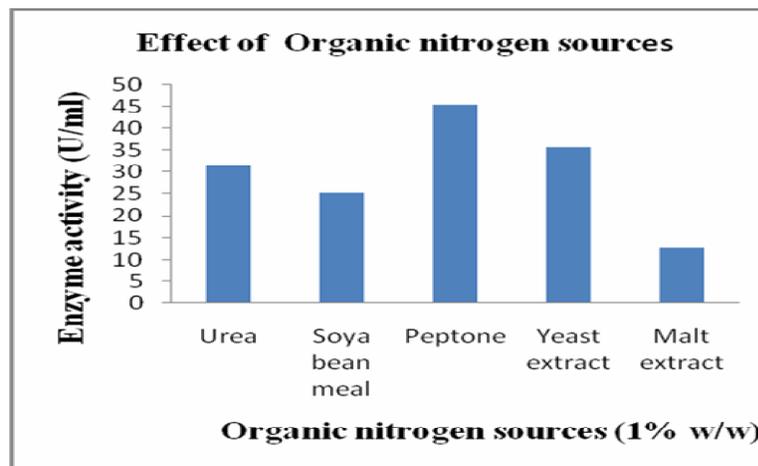


Fig.3 Effect of Organic nitrogen supplements on glucoamylase



Of the five different organic nitrogen supplements used as enrichment, ammonium sulphate was a good inorganic supplement which gave maximum glucoamylase activity 32.25 U/ml (Figure 4) but when compared with organic supplements it is lower therefore inorganic nitrogen supplements has no effect on glucoamylase activity.

Glucoamylases are the most widely used enzymes in industries. In the present work, four different substrates and microorganisms were screened. It was found that, maximum activity of glucoamylase 36.31U/ml was found using wheat bran with *A.oryzae* NCIM1212. Laboratory scale optimization of some of fermentation conditions for glucoamylase production by *A.oryzae* NCIM 1212 in SSF was carried out. The chemical parameters like carbon and nitrogen supplements. The nutritional supplementation study was carried out using various carbons, nitrogen (organic and inorganic supplements) to enrich the production medium to enhance the enzyme yield. The optimized conditions are: Carbon source: Addition of Fructose as carbon source showed high glucoamylase activity of 42.32 U/ml compared to other carbon sources like glucose, maltose, sucrose, and soluble starch. Nitrogen source: Addition of peptone as organic nitrogen supplement showed high glucoamylase activity of 45.21U/ml than other nitrogen sources like yeast extract, malt extract, urea and soya bean meal. Addition of Ammonium sulphate as inorganic nitrogen supplement showed reduced glucoamylase activity. As well as optimization parameters carried out in this study proved to be fruitful in enhancing programs for enzymes of biotechnological importance.

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