Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1457-1461, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

OPTIMIZATION OF SOLID STATE FERMENTATION FOR THE PRODUCTION OF GLUCOAMYLASE FROM *ASPERGILLUSSPECIES* USING AGRO WASTESUBSTRATE

[1,*]Shantha, [2]Revathi chitra, [3]Rega and [4]Arockia Badhsheeba

Department of Biotechnology, Kumararani Meena Muthiah College of Arts and Science, Adyar, Chennai-600020, Tamil Nadu, India

Received 24th February, 2018; Accepted 28th March, 2018; Published Online 06th April, 2018

ABSTRACT

Aspergillus species is one of the most suitable species to grow in the solid substrate for maximum glucoamylase production. The enzyme titres varied considerably in different substrates. The particle size and the chemical composition of the substrate influence the fungal growth and enzyme production. The aim of our study is to analyze the biochemical contents present in the selected substrates (Rice bran, wheat bran and tea waste) for glycoamylase production and to optimize the moisture content in the selected substrates to enhance glucoamylase production and also to find out the effect of various physical and biochemical parameters to maximize glucoamylase production. From the study it was found that good production of glucoamylase from *Aspergillus niger* and *Aspergillus flavus*species was found in 65 to 75% of moisture content were the pH 3 and pH 9 with the optimum temperature of 45°C.Among the different solid state substrates used nitrogen source of 1% peptone and 1% yeast extract accelerated the enzyme productionand it was also noted that 1% maltose and 1% starch showed higher enzyme production.The produced glucoamylase enzyme was finally eluted using the column chromatography. The eluted glucoamylase can be used for industrial product production like fermented foods.

Key words: Glucoamylase, Solid State Fermentation, *Aspergillus niger, Aspergillus flavus* and column chromatography.

Citation: Shantha, Revathi chitra, Rega et al., 2018. "Optimization of solid state fermentation for the Production of glucoamylase from *Aspergillusspecies* using agro wastesubstrate" *International Journal of Current Research in Life Sciences*, 7, (04), 1457-1461.

INTRODUCTION

Solid State Fermentation involves the growth and fermentation by micro organisms, especially fungi, on moist, water in soluble Solid substrate in the absence of free flowing water. The water content is quite low with the required moisture existing in complex form with in the Solid material so that microorganisms are almost not in constant contact with gaseous oxygen but also serves as an anchorage for the microbial cells. The ideal solid substrate is one that provides all necessary nutrients for the growth of micro organisms. Production of concentrated enzyme preparations could be obtained more by Solid State Fermentation than by submerged fermentation. New applications are currently gaining momentum such as production of enzymes and feed additives. Some of the salient features of solid state fermentation in enzyme production are less requirements of space with simple equipments, utilization of cheap raw materials and less pollution hazards. Glucoamylase is a glyco protein containing mannose, glucose, galactose and uronic acid and has a molecular weight of $60X10^3$ to $100X10^3$.

Corresponding author: **Shantha,**
Department of Biotechnology, Kumararani Meena Muthiah College of Arts and science, Adyar, Chennai-600020, Tamil Nadu, India.

Glucoamylase catalyse the stepwise hydrolysis of 1-1.4 links in starch and oligo saccharides releasing β- glucose molecules from the non reducing sugars. Solid state fermentation a process that occur in the absence of free water has advantages over Sub-Merged fermentation Some physical, cultural and chemical parameters influences the production of ghucoamylase. Several authors have described the protocol for purification and properties of glucoamylase. In many instances, multiple forms of glucoamylase have been reported (Line back *et al.,* 1972). Commercial preparations of glucoamylases were purified by ultra filteration and sepharose gel filteration. Two forms of the glucoamylase I & glucoamylase II are commonly obtained from *Aspergillus* species. The main advantage of Solid state fermentation is the ease with which fungi can grow on complex natural solid state substrates like agro – industrial residues. There are some important properties like macro molecular structure, size, shape, consistency of particle and porosity of a substrate which need to be considered for use in Solid state fermentation. The suitability of wheat bran as the substrate for Solid state fermentation can be attributed to its increased surface area due to pretreatment, pore size and nutrient availability. A number of moistening agents have been used in Solid state fermentation. Moisture content of the fermentation medium is one of the main factors that often determine the success of the process. Hence a study was carried out to optimize the culture

conditions for glucoamylase production using *Aspergillus* species by solid state fermentation.

MATERIALS AND METHODS

Collection of Substrates

The agro wastes such as rice bran, wheat bran, tea waste were collected and dried.

Cultures Used

Aspergillus species such as *Aspergillus niger* and *Aspergillus flavu*s was maintained through successive transfer on czepeckdox mediumand 1mL of a spore suspension (10^7 spores/mL) prepared was used as the inoculum, Raw starch activity was measured to this selected cultures. 2% agar in phosphate buffer was prepared to the sterile plate and inform diameter of well was made. To this well lml of culture filtrate was added and incubated at 37ºC for overnight and hydrolysis was visualized by the addition of 0.01N Iodine solution.

Parameters Analyzed In Substrates Before Inoculation

The raw substrates used for the study were analyzed for carbohydrate, reducing sugars and protein.

Estimation of Carbohydrate: (Anthrone Method)

Carbohydrates were dehydrated with concentrated H_2SO_4 to form furfural. This furfural condenses with anthrone to form blue colour complex which was measured spectrophotometrically. Total Carbohydrate was estimated by the anthrone Method by Sadasivam and Manickam (1992). 100mg of the sample was taken and to this 5mL of cold anthrone reagent was vortexed rapidly. The tubes were covered with aluminum foil and kept in a boiling water bath for 10 minutes and then cooled to room temperature. The absorbance was read at 625nm using spectrophotometer and standard graph was made using glucose as standard and total carbohydrate of the sample was calculated from the standard graph.

Protein Estimation: (Lowry's Method)

Protein content was analysed by Lowry Method described by Lowry *et al.,* (1951). 0.2g of sample was mixed with water and centrifuged at 2000rpm for 20m. lmL of the supernatant was taken and to this 5ml of freshly prepared alkaline copper sulphate solution (reagent – C) was added and incubated for 10m. And then 0.5mL of Folin Phenol reagent was added and incubated for 30m. The absorbance was read at 660nm using spectrophotometer. A blank containing 0.1mL of NaOH and all the other reagent were added to adjust the absorbance to zero and the protein content of the sample was calculated from the standard graph.

Estimation Of Reducing Sugar: (Dns Method)

100mg of substrates was mixed with 80% of ethanol and allowed it to centrifuge at 3000rpm for 15m. To 2mL of the supernatant 3mL of DNS reagent was added and kept the tubes in a boiling water bath for 15m. The reaction was stopped by the addition of 4mL of distilled water and OD was measured at 540nm.

Determination of Optimization of Moisture Content For The Production Glucoamylase Using *Aspergillus Niger* And *Aspergillus Flavus*

Commercially available Rice bran, Wheat bran, Tea waste was used as solid substrates. The substrates of varying moisture levels (45%, 55%, 65%, 75%) for the optimization glucoamylase production, was tested and the optimum moisture content for *Aspergillus niger* and *Aspergillus flavus* was determined in all the substrates.
The moisture content was fixed for both the organisms namely *Aspergillus niger* (65%) and *Aspergillus flavus* (75%) and further analysis were done having this concentration of moisture content.

Effect of Carbon Sources on Glucoamylase Production Using*aspergillus Niger* And *Aspergillus Flavus*

Using fixed moisture content, the carbon sources such as dextrose, fructose, maltose, and starch at 1% concentration were applied individually in the place of sucrose in the czepeckdox medium. After adjusting the moisture content to fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture were inoculated and incubated at 37^{o}C for 48hrs and the enzyme production was analyzed.

Effect of Nitrogen Source on Glucoamylase Production Using *Aspergillus Niger* And *Aspergillus Flavus*

Using fixed moisture content, the nitrogen source namely Ammonium nitrate, Peptone, Tryptone and yeast extract at 1% concentration were supplemented in each treatments. After adjusting the moisture content to fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture were inoculated & incubated for 48hrs and enzyme production was analyzed.

Effect of Ph on Glucoamylase Production

The substrates at different range of pH (such as pH3, 5,7 and 9) were used. Using the fixed moisture content after adjusting the pH and moisture content to a fixed level, the substrates were autoclaved at 15lb for 20m. A loopful of culture was inoculated to the substrates & incubated at 37^{o}C for 48hrs and the production was analysed.

Effect of Temperature on Glucoamylase Production

Using fixed moisture content of the substrates, the substrates were autoclaved at 15lb for 20m and a loopful of culture were inoculated to the substrates and incubated at different temperature such as 35^{o}C, 45^{o}C, 55^{o}C, and 65^{o}C.

Effect of Inoculum Size on Glucoamylase Production

0.1mL of culture was taken and dropped into a clean haemocytometer, and the total number of spores in the A.niger and *Aspergillus flavus* cultures was counted, and the values were noted respectively. After adjusting the moisture content to a fixed level, the substrates were autoclaved at 15lb for 20m of the culture. The *Aspergillus niger* suspension was inoculated as $3x10^8$, 6x, $9x10^8$, $12x10^8$ the *Aspergillus flavus*

suspension was inoculated as $4x10^8$, $8x10^8$, $12x10^8$ respectively.

Enzyme assay

To lmL of culture filtrate, 32mg of soluble starch in 0.1M citrate buffer was added and incubated at 50-60^oC for 30m 0.5mL of enzyme substrate mixture was taken and 2mL of DNS was added and kept the tubes in a boiling water bath for 5mins and cooled. The volume was made to 5ml of distilled water, and the enzyme was analysed spectrophotometrically.

Elution of glucoamylase using column chromatography

Two volume of cold acetone (-10^oC) was added to the culture filtrate and the pellet was removed by centrifugation and dissolved with 150ml of 0.02M Phosphate buffer (pH 7.0). This solution was applied to a DEAE cellulose column (diethyl amino ethyl cellulose column). Washing of DNAE cellulose was done by 10g of DEAE cellulose containing 200mL of lM NaCl and it was stirred vigorously for a few minutes and filtered through a WHATMAN No.1 filter paper and was suspended the materials in 200mL of phosphate buffer.
The column tube (2.5x2.5cm) was set on a burette stand vertically. The suspension DEAE cellulose was powdered into the column gently through the sides, avoiding trapping of air bubbles. Simultaneously; the column outlet was opened so that the adsorbent settle down. Then the column was equilibrated with phosphate buffer and the column was eluted with a linear gradient of NaCl (0.1M to 0.5M) with a flow rate of 0.7mL/min. From this, 5mL of fraction was collected and the fraction containing glucoamylase activity was pooled together and used for various propose.

Immobilization of the enzyme

was done using 7mL of 2.5% (W/V) sodium alginate added to the enzyme solution and the solution was allowed to stand for 30mins. Then 7mL of 2.5% glutaraldehyde was added and kept it for 11/12hrs and this solution was added drop wise into 0.05M $CaCl_2$ solution and the beads were collected and stored at 4^oC.

RESULT AND DISCUSSION

Starch hydrolyzing activity in *Aspergillus niger*and *Aspergillus flavus*

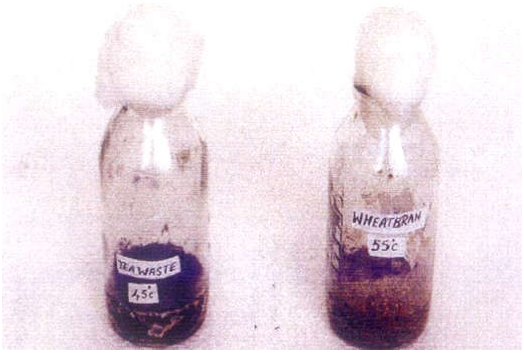
Glucoamylase production was carried out by selected agro wastes using fungal organisms such as *Aspergillus niger*, and *Aspergillus flvaus*It was noted that better results was observed in *Aspergillus.niger* culture than in *Aspergillus flavus*

Biochemical analysis of raw substrates

The proteins and reducing sugar content was found to be higher in rice bran (0.36mg/mL, 0.099 mg/mL) whereas the Carbohydrate content was more in wheat bran (2.632 mg/mL) than the other two substrates . The production of glucoamylase was high when wheat bran and rice bran used as a substrate than the tea waste. Hence rice bran and wheat bran are considered to be ideal substrates for glucoamylase production.

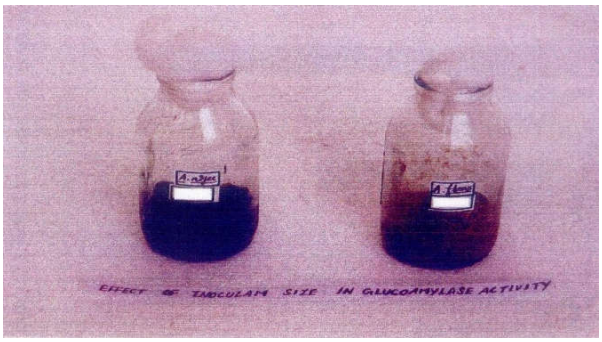
Effect of temperatue on glucoamylase production in solid state fermentation using *Aspergillus niger* and *Aspergillus flavus*

High glucoamylase production was observed at pH of 3 and pH9 in *Aspergillus niger* and *Aspergillus flavus* respectively. Temperature 45^oC and 55^oC yields maximum glucoamylase activity in both the organisms. The augmentation of inoculums size $3x10^8$ and $16x10^8$ (spores/mL) influence the glucoamylase activity both in *Aspergillus niger* and *Aspergillus flavus*.



Effect of inoculums size on the production of Glucoamylase using *Aspergillus niger* and *Aspergillus flavus*

With regard to the effect of inoculums size on the enzyme production, it was observed that S3 treated with $3x10^8$ and $9x10^8$ amount of inoculums produced maximum amount of enzyme when *Aspergillus niger* was used. Similarly *Aspergillus flavus* was used, it was found that in S3 and S1 with $8x10^8$& $16x10^8$ amount of inoculums enhanced the production of glucoamylase.

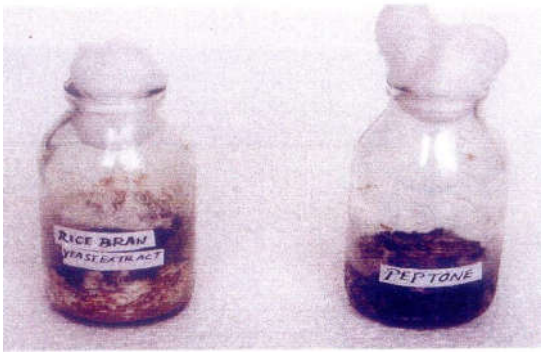


Effect of Fructose and Maltose on glucoamylase production in rice bran and wheat bran using *Aspergillus niger* and *Aspergillus flavus*

The present study rice bran (S1) and tea waste (S3) also produced high enzyme production when starch was used as the carbon source. Same results were observed in *Aspergillus flavus*.High enzyme activity exhibited when dextrose, maltose and starch were used as the carbon source in which, maximum production was noticed in S3 and in S1. Poor enzyme activity was observed in the fructose treated substrate.

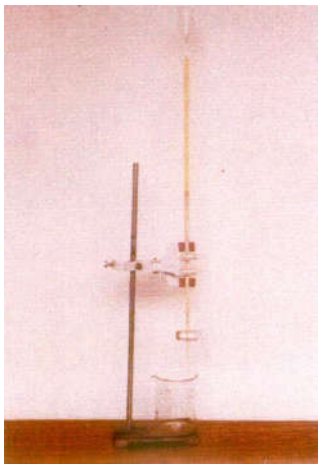
Effect of yeast extract and peptone on glucoamylase production in rice bran and Tea waste using *Aspergillus niger* and *Aspergillus flavus*

High glucoamylase production was found in the substrate supplementation of 1% fructose and 1% of yeast extract.

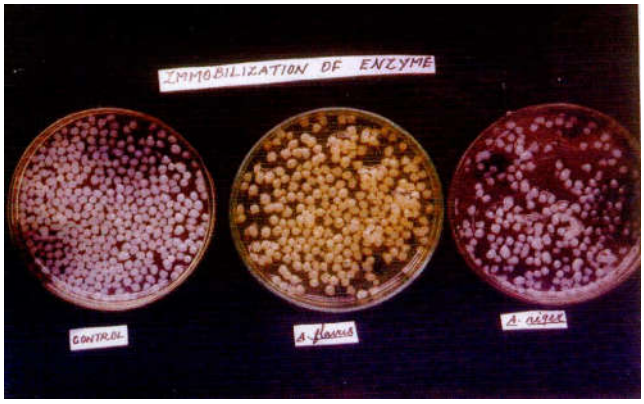


Elution of Glucoamylase using 0.1-0.5 m $CaCl_2$ column chromatography

It was found elution of enzyme production was high when 0.3M and 0.5M CaCl2 were used in *A.niger* treatments. In contrast, only low enzyme was eluted in *A.flavus* treatments using 0.3M and 0.4M CaCl2. Experiments on elution of glucoamylase enzyme with 0.1M concentration of $CaCl_2$ in column chromatography also showed higher glucoamylase activity.



***Im*mobilization of Glucoamylase using $CaCl_2$**



It was found that 65% and 75% of moisture content was found optimum for *Aspergillus niger* and *Aspergillus flavus* for glucoamylase production.

REFERENCE

Adinarayana Kunamneni, Kugen, 2005. Production in solid state fermentation by the *Thermophilic fungus, and Thermomycetes lanuginosus, Journal of bioscience and BioEngineering*. 100 (2): 168 – 171.

Babu K.R, Satyanarayana T, 1995. Parametric optimization of extra cellular α-amylase production by the *Thermophilic Bacillus* coagulans. *Journal of Folia Microbiology*. 38: 7780.

Cadmum, J.A.V., Alegre, R.M., and Hasan, 1996. Packing density and thermal conductivity determination for rice bran solid state fermentation. *Biotechnology. Tech.*12: 747-750.

Evelyn, M., Doyle, Cathe Rine, T., Kelly, and Willum, 1994. Alpha and glucoamylase production using aspergillus species in Solid state fermentation. *Journal Applies Microbiology and Biotechnology*, 30: 492-496.

Fogarty, W.M., Kelly, C.T., 1995. Starch degrading enzyme of microbial origin. *Progress in industrial microbiology.*15: 87–150.

Hema Anto, *et al.,* 2005. Ujjval Trivedi and Kamlesh Patel, (2004). Alpha amylase production by *Bacillus cereus* MTCU 1305 using SSF. *Journal of Food Technology and Biotechnology*. 44(2): 241–245.

Hussain, K., Surangzeb Baig, M.A., Ahemed, R., Khan, J., (1994), Production of glucoamylase from agrowastes by *Rhizopus nigricans*. *Sachard Journal of Agricultural Microbiology*10: 623 –628.

Jensen, and Nelson, (1991). The influence of nitrogen source on alpha amylase productivity of *Aspergillus oryzae* in continuous cultures. *Apllied Microbiology. Biotechnology.*53: 271.

Kawmura, and Sawai, 1966. Production of glucoamylase by *Aspergillus awamori* using wheat in submerged and solid state fermentations. *Indian journal of Microb*iology. 35(5): 123-125.

Larry, L., Barton Carl, E., Georgi, and Devid, R., Line back, 2005. Effect of maltose on glucoamylase formation by *Aspergillus niger, Journal of Bacteriology*. 111(3): 771-777.

Lineback, R., David, H., (1972). Effect of maltose on glucoamylase formation by *Aspergillus niger.Journal of Bacteriology.*65: 771-777.

Mishra, A., Debnath Das, M., 1993. Production of glucoamylase by *Aspergillus oryzae* MTCC 152 in SSF. *Indian Journal of microbiology*ISSNO0046 – 8991.

Mortia, and Wihinen, M., Mentsala, P., 1966. Production of Microbial amylolytic enzymes. *Journal of Biochemsitry Molecular Biology.*246: 329-410.

Parminder Kawr, H., Grewal, S., and Kocher, G.S., (2003). Production of α-amylase and glucoamylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations, *Indian Journal Of Microbiology*. 43(2): 143-145.

Pazur, J.H., Tominaga, and Anto, 1959. Glucoenzymes: an unusual type of glycoprotein structure for a glucoamylase. *Carbohydrate. Res.*84: 103-114.

Priert, F.G., 1984. Extracellular enzymes. *Aspects of Microbiology*. 9: 223-227.

Production by Aspergillus awamori in SSF. 58: 708-712.

Ramesh M.V. and Lonsane b.K. 1990, Production of bacterial thermostable alpha amylase by SSF. A potential tool for achieving economy in enzymes production and starch

hydrolysis. *Journal of Adv. Applied Microbiology*, 35: 1-47.

Stailova, I.S., Gargova, S.A., Krastanov, A, I., 2005. Production of enzymes by mixed culture from micelial fungi in SSF. *Journal of Biotechnology*. 1: 103-108.

Walkar, N.E., Campbell, L.L., 1983. Effect of carbon souces on formation of α- and glucoamylase by *Bacillus stearothermophiles*. *Journal of Bacteriology*. 86: 681-686.

****