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RESEARCH ARTICLE

PRODUCTION, CHARACTERIZATION, KINETIC STUDIES OF GLUCOAMYLASE THROUGH SOLID-STATE FERMENTATION BY ASPERGILLUS NIGER USING AGRICULTURAL RESIDUES AS SUBSTRATE

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ABSTRACT

In the current study the Glucoamylase production was carried out by *Aspergillus niger* on the solid state fermentation of Agricultural residues. The Agricultural residues, subjected as the substrate for glucoamylase production are Ricebran, Cotton seed flour, Coconut oil cake, Wheat bran, Groundnut oil cake, each in a separate conical flask. *Aspergillus niger* was incubated in those different agro residues for 5 days at 37°C for finding the suitable substrate favouring the better enzyme expression with optimum characteristics features. The cultural conditions were optimized for the enzyme production, 20% [50/250ml-flask] was found to be optimum volume of the medium. With comparison of all the five agro residues "Ricebran" showed high yield of (7µg/ml) Glucoamylases in SSF. Optimum enzyme activity was observed at 45 °C at pH 5.2. The kinetic studies of Michaelis Menton parameters includes the estimation of V_{max} and K_m, were 20(µg/ml.min), 10(g/l).

INTRODUCTION

Glucoamylase is an enzyme hydrolyzes single glucosidic residues from the non-reducing ends of amylose and amylopectin in a stepwise manner. GA also hydrolyses the 1,6-alpha-glucosidic linkage in the branching point of amylopectins, although at a slower rate than the alpha-linkages (Pandey, 1995). Glucoamylase involving in a multi-step process of starch saccharification at pH 6.0 and 60°C. GA is mostly produced by molds, mainly *Aspergillus sp*. Production is generally extracellular and enzyme can be recovered from culture filtrates. Traditionally, GA has been industrially produced by Submerged fermentation (SMF); however, in recent years, Solid state fermentation (SSF) has also been considered promising for GA production [1]. SSF processes are selected when the substrate is solid or starchy. That is, SSF is generally defined as the growth of micro organisms on solid materials [2]. No water solution is present but water is also associated with the solid components. However, the substrate must contain enough moisture, which is provided by evaporation and metabolic activity [3] .For this, agro-industrial residues are generally considered good substrates (Ellaiah *et al*. 2002) some examples include wheat bran, rice bran, ricehusk, gramflour, wheatflour, cornflour, tea waste, etc (Pandey and Radhakrishnan1992,1993) [1] From economic points of view, some advantages of SSF over SMF, can be described as: a- Less energy consumption in fungal −α amylase production, b- The longer production phase in amyloglucosidase production, c- The shorter fermentation period in catalyses production, d- The use of waste or spent wheat bran in cellulase and production, e- The extra-cellular nature of the enzyme in –α galactosidase production, f- No foam generation [4], industrially important hydrolytic enzymes of biotechnological significance and are currently used in food and pharmaceutical industries (Joshi *et al*. 1999). Glucoamylases mainly used in the production of glucose syrup, high fructose corn syrup, and alcohol (James and Lee, 1997 [5]. The main objectives of present study was to make use agro residues available as the cheaper source for the effective enzyme production. This paper reports selection of the five different form of the agro residues, Rice bran, Cotton seed flour, Coconut oil cake, Wheatbran, Groundnutoilcake. Then finding out which substrate complement better enzyme expression for the Commercialization of the process in fermentation technology.

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MATERIALS AND METHODS

Microorganism

The culture type of *A. niger* was obtained from the PRIST University (East campus). Stock cultures were maintained on PDA (potato-dextrose-agar) medium. The cultures were preserved at 4 °C renewed once a month.

Substrates

The agricultural residues selected as substrate for the solid state fermentation comprise of Rice bran(Sample-1), Wheat bran (Sample-2), Cotton seed flour,(Sample-3),Coconut oil cake (Sample-4), Ground nut oil cake (Sample-5) were

collected from local market at Tanjore and preserved at room temperature**.**

Solid state fermentation

Experiments in flasks

Agro residues (20 g) of each sample were kept in a separate 250 mL Erlenmeyer flask and then moistened with 50% of water and sterilized at 121 °C for 30 min [6]**.** The fermentation process was started by adding one mL of spore suspension (5 x 10^7 spores/mL) as prepared above [5]. The whole content was mixed thoroughly and then incubated at 37° C for 5 days in a Static condition.

Enzyme extraction

To the fermented dough 50 mM citrate buffer (pH 5) (1:10) was added and homogenized for 2 h 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 [21]. This solid-free supernatant was used as enzyme source for assaying glucoamylase activity.

Enzyme assay

Glucoamylase activity was measured by; 5 ml of 0.1% soluble starch, 1ml of 0.1M acetate buffer at pH=5, 1 ml of D/W and 1 ml of enzyme solution were incubated at 40 $^{\circ}$C in water bath for 10min. The reaction was stopped by addition of 0.5ml dinitrosalicylic acid reagent. The absorbance was recorded at 540 nm [12].

Michaelis-Menton Kinetics of enzyme activity for different Agro residues

AGRO RESIDUES	Vmax (µg/ml.min)	Km (g/l)
WB	12.1	6.05
GOC	15	7.5
RB	20	10
CSF	19	9.5
COC	18	9

Protein Concentration of enzyme activity for different Agro residues

Agroresidues	Protein Concentration(µg/ml)
COC	4
CSF	3
RB	7
WB	2
GOC	3.5

Characterization and Kinetic studies of Glucoamylase

The purified glucoamylase was subjected to characterization through kinetic studies by studying the following:

- Effect of pH on glucoamylase
- Effect of temperature
- Effect of substrate concentration, determination of Km and Vmax.

Protein estimation

Protein was estimated using protein-Lowry's method. For this purpose, different concentrations of bovine serum albumin (BSA) were prepared and were run on the spectrophotometer to take the absorbance [12]. A standard curve of the protein was prepared from the absorbance shown by standard solutions of BSA.

RESULT AND DISCUSSION

The production of extracellular glucoamylase by *A. niger* was studied in solid state fermentation (SSF). In the present study five different substrates, like Rice bran (Sample-1), Wheat bran (Sample-2), Cotton seed flour (Sample-3), coconut oil cake (Sample-4), Groundnut oilcake (Sample-5) were used for glucoamylase production[4].Total of 5 agro residues were screened. RB gave the highest enzyme production followed by WB. Wheat sbran as the most promising substrate for glucoamylase production has been reported by several researchers (Kaur *et al.* 2003; Anto *et al* 2006; Pandey *et al* 1999). Production of very high levels of a hard starch-gel digesting glucoamylase under SSF using wheat bran, rice bran, and other components of these has been reported (Singh and Soni, 2001) [13].

Effect of pH

Aspergillus niger was inoculated into different substrates were incubated at room temperature for five days. The enzyme was extracted and the specific activities of the Glucoamylase produced at different pH and in different substrates were recorded (Figure: 1).The maximum activity of Glucoamylase was in pH 5 and the pH of wheat bran was 4.5. This was very high when compared to other pH range [12]. Effect of pH on the medium is shown in Fig.1. The maximum glucoamylase production was obtained at pH=5.0 after 5 days of incubation at37 $^{\circ}$C. Optimum pH is very important, the composition of cell wall and plasma membrane of microorganism is known to be affected by the culture pH**.** Optimization of the culture conditions for glucoamylase production by *Aspergillus sp* under SSF and optimum enzyme yield noted at pH=5 **.**

Effect of Temperature

Aspergillus niger when inoculated at different temperature 35 $^{\circ}$C, 40 $^{\circ}$C, 45 $^{\circ}$C , 50 $^{\circ}$C, and 55 $^{\circ}$C showed maximum yield of Glucoamylase at 55 $^{\circ}$C in Rice bran. There was increase in yield in other agro residues when the temperature was 45 $^{\circ}$C Growth temperature is a very critical parameter which varies from organism to organism and slight changes in growth temperature may affect glucoamylase production. Organisms have various mechanisms that allow them strictly to control excretion.

Enzyme Kinetic Parameters

The Kinetic Parameters of the enzymatic activity using starch as a reaction limited substrate could be expressed using simple Michaelis-Menton Kinetics (Fig.3) expressed by equation. The resulted plot has a slope equal Km/Vmax (Km = substrate saturation constant and Vmax = maximum velocity) an intercept equal 1/Vmax. This result showed that enzyme used in this study had a higher catalytic activity for starch. From the kinetic studies, it can be concluded that is industrial potential is high. The kinetic parameters of glucoamylase activity give remarkable value of industrial applicability. The obtained results indicate that *Aspergillus niger* has potential for the

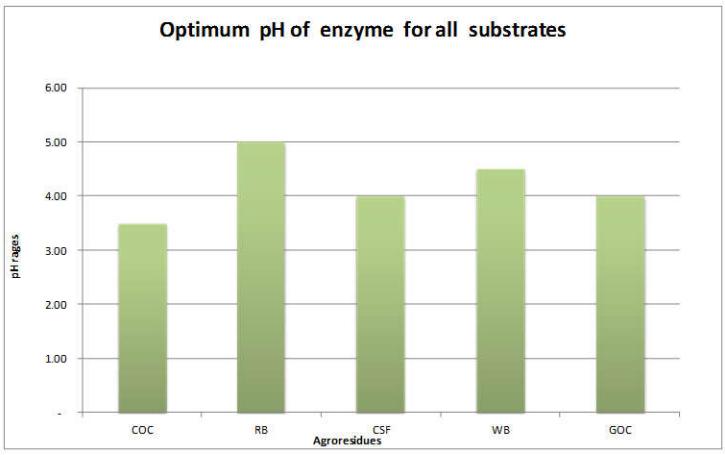

Fig. 1: Optimum pH of enzyme activity for different Agro residues

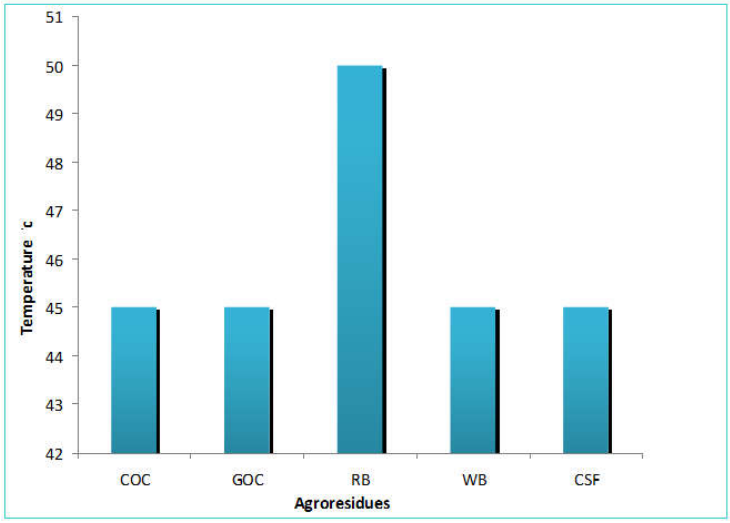

Fig. 2: Optimum temperature of enzyme activity for different Agro residues

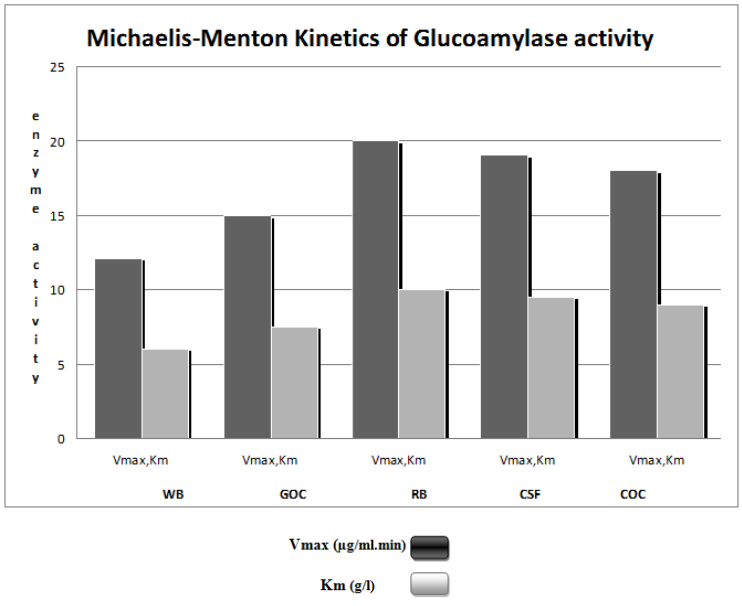

Fig. 3: Michaelis-Menton Kinetics of enzyme activity for different Agro residues (WB, GOC, RB, CSF, COC)

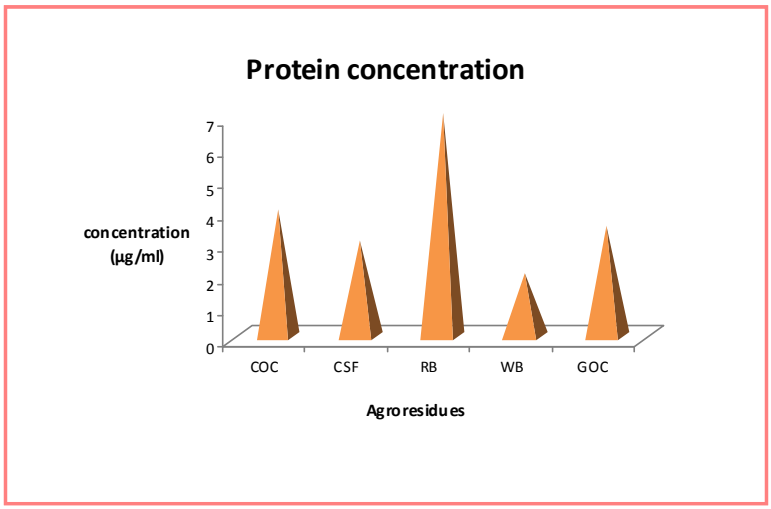

Fig. 4: Protein Concentration of enzyme activity for different Agro residues

production of glucoamylase of relatively improve stability at different conditions.

Conclusion

It has been demonstrated that *Aspergillus niger* has the potential to utilize agricultural waste residues for production of glucoamylase enzyme. The higher glucoamylase activity can be obtained on inexpensive and easily available substrate - Ricebran by *A. niger* in SSF.SSF offers numerous advantages over submerged fermentation; these include high productivity, relatively higher concentration of products, less effluent generation and simple fermentation equipment. Thus Rice bran residue could be a potential, economic source for the production of gluco-amylase by solid state fermentation.

REFERENCES

[1]. Pandey, A., "Recent process developments in solid-statefermentation", *Process Biochemistry,* Vol. 27, (1992), 109-117.

[2]. Baysal, Z., Uyar, F. and Aytekin, C., "Solid State Fermentation for production of Alfa-Amylase by a thermotolerant Bacillus subtilis from hot-spring water", *Process Biochemistry,* Vol. 38, (2003), 1665-1668.

[3]. Bertolin, T.E., Schmidell, W., Maiorano, A.E., CasaraJ.and Costa, J.A.V., "Influence of Carbon, Nitrogenand Phosphorous Sources on Glucoamylase Production by *Aspergillusawamori*in Solid State Fermentation", *Zeitschrift fur Naturforschung*, Vol. 58, (2003), 708-712.

[4]. Pandey, A., "Aspects of fermentation design for solidstate fermentation", *Process Biochemistry,* Vol. 26, (1991), 355-361.

[5]. James JA and Lee BH (1997) Glucoamylases: microbial sources, industrial applications and molecular biology: A review. *Journal of Food Biochemistry*, 21: 1-52.

[6]. Zadrazil, F. and Brunnert, H., "Solid-state fermentations", *European Journal of Applied Microbiology and Biotechnology*, Vol. 11, (1981), 183-188.

[7]. Pandey,A., Selvakumar, P. and Ashakumary, L., "Performance of a column bioreactor for glucoamylase synthesis by *Aspergillus niger* in SSF", *Process Biochemistry,* Vol.31, (1996), 43–46.

[8]. Ramachandran, A. K. Patel, K.M. Nampoothiri, S. Chandran, G. Szakacs, C.R. Soccoland, A. Pandey (2004) *Braz. Arch. Biol. Technol*. 47, 309-317.

[9]. Gertler and Yehudith Birk (1965) *Biochem. J.* 95, 621-627.

[10]. Bernfield P. (1995) Amylase α and β. In: Methods in Enzymology, Vol.1, Academic Press, New York, USA, 149-158.

[11]. Bilinski and Stewart. Production and characterization of α-amylase from *Aspergillus niger*, 1995; 18: 551-556.

[12] Gupta A, Gupta VK, Modi DR, YAdava LP. Production and characterization of α-amylase from *Aspergillus niger*. Biotechnology 2008; 7: 551-556.

[13]. Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P, Srinivasulu B (2002). Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. Process Biochem. 38: 615-620.

[14]. Kaur P, Grewal HS, Kocher GS (2003). Production of α-amylase by *Aspergillus niger* using wheat bran in submerged and solid state fermentations. Indian J. Microbiol. 43:143-145.

[15]. Ramachandran, S., Patel, A. K., Nampoothiri, K.M., Francis, F., Nagy, V., Szakacs, G., et al. (2004a). Coconut oil cake—a potential rawmaterial for the production of a-amylase. Bioresource Technology, 93, 169–174.

[16]. Ramachandran S, A.K. Patel, K.M. Nampoothiri, S. Chandran, G. Szakacs, C.R. Soccol, Pandey A, (2004b). Alpha amylase from a fungal culture grown on oil cakes and its properties, *Braz. Arch. Biol. Technol. 47:*309–317.

[17]. Vasudeo Zambare (2010). Solid state fermentation of *Aspergillus oryzae* for glucoamylase production on agro residues, International Journal of Life Science 4:16-25.

[18]. Pandey A. (1995) Glucoamylase research: An overview, Starch/ Starke, 47, 439-445.

[20]. Pandey A., and Radhakrishnan S., "The Production of Glucoamylase by *Aspergillus niger* NCIM 1245", *Process Biochemistry,* Vol. 28, (1993), 305-309.