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Research article

POTENTIAL OF SOIL MYCOTIC FLORA FOR BIO-CONVERSION OF CHEAP AGRICULTURAL RESIDUES

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ABSTRACT: The fungal glucoamylase production using agricultural residues presents great potential for its use in various industrial purposes. In the present study, soil fungi were used for the production of glucoamylase by solid state fermentation using agricultural residues (rice bran, wheat bran, wheat straw and rice husk). Solid state fermentation was carried out at temperatures (20°C, 27°C, 37°C, 45°C and 60°C) , pH (4.5, 6, 7.5, 8 and 9.5), incubation time (regular interval for 5 days), ratio of moisture level in the basal media (1:1,1:2,1:3,1:4,1:5).The results showed that rice bran with moisture level at the ratio of 1:4 at temperature27°C, pH 6and 3days of incubation was best for optimum production of glucoamylase. The study shows the use of cheap, readily available agricultural residues for the low cost production of glucoamylase. This alternative strategy can be exploited in various industrial applications of biotechnological importance.
Key words: Glucoamylase, Agricultural residues, Solid state fermentation, Biotechnological important.

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INTRODUCTION

The enzymes from microbial sources are generally used for industrial purposes. In recent years; the potential of using microorganisms as biotechnology sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Abu et al., 2005).Starch is one of the most important naturally occurring glucose polymers and used as the starting material for glucoamylase production. The carbon sources such as dextrin, fructose, glucose, lactose, maltose and starch are very expensive for commercial production. Various agricultural byproducts are abundantly available for the bioconversion to products of economic importance. The bioconversion of these agriculture byproducts to enzymes like glucoamylase offers an alternative for their utilization (Parbat and Singhal, 2011).

Glucoamylase (α -1,4glucan glucohydrolase, amyloglucosidase) is of great importance to the fermentation and food industries for saccharification of starch and other related oligosaccharides. Glucoamylase (GA) consecutively hydrolyzes α -1, 4 glycosidic bonds from the non-reducing ends of starch, resulting in the production of glucose. It also hydrolyzes α-1, 6 linkages but to lesser extent (Pandey et al., 2000). Glucoamylase occur almost exclusively in fungi (Sandhu et al., 2015) and especially *Aspergillus niger*, *Aspergillus awamori*, *Rhizopus oryzae* and *Fusarium* species have been considered the most important for industrial application.

The solid state fermentation (SSF) process has the potential to significantly reduce the enzyme production costs because of lower energy requirements, increased productivity, smaller effluent volumes and simpler fermentation equipment (Zambare, 2010).The different factors affecting SSF includes moisture, water activity, pH, temperature and substrate. The agro industrial byproducts like Wheat bran, Rice bran, Rice husk, Corn flours, Cereal flours, Cereal bran, potato residue, oat bran,sugarcane baggase and other starchy waste materials are considered for production of glucoamylase (Parbat and Singhal, 2011).

The glucoamylase production by solid-state fermentation was studied using rice flake manufacturing waste products as substrate. Wheat bran, paddy husk rice processing waste and other starch containing waste have gained importance as supports for growth during enzyme production (Anto et al., 2006).Also, theglucoamylase production was studied by *Aspergillus oryzae*via solid state fermentation on Agro residue (Zambare, 2010).

It has been reported that the isolation and screening of amylase and glucoamylase producing fungi and their application in bioethanol production has been studied earlier (Ominyi, 2013).The glucoamylase production under solid state fermentation using low cost byproducts of agricultural processes as substrate was reported (Nahid et al., 2011). Also in few reports, the screening of four agricultural residues viz. rice bran, wheat bran, rice bran: wheat bran (1:1), rice bran: paddy husk (1:1) (Puri et al., 2013).

The present study focuses on the bioconversion of agricultural residues for glucoamylase by soil fungal isolates. Studies were also conducted for the determination of moisture level, temperature,pH and incubation time for solid state fermentation of agricultural residues.

MATERIALS AND METHODS

Screening of fungal isolates

Soil sample collected from different places of Jabalpur in a sterile container was brought to the laboratory for further processing. It was serially diluted up 10^{-6} dilution by the serial dilution method (Wahab et al., 2012).All the fungal colonies were examined for amylase production by starch hydrolysis test. The fungal isolates were inoculated on starch agar media and then plates were incubated at 28^{o}C for 2-3 days. After incubation, the plates were flooded with iodine solution and examined for starch hydrolysis (Abu et al., 2005).

Solid state fermentation using agro-industrial waste

In the present study, the pre-treatment of agro-industrial residues like rice bran, rice straw, wheat bran and wheat straw was done by their collection from the agriculture fields near Jabalpur region. The residues were washed with cold water and warm water for the removal of dust particles and impurities. These were then air dried and kept at 50ºC.

Solid state fermentation was carried out by using basal media which consist of solid substrate moistened with mineral media. The flask containing basal media with substrates was autoclaved, cooled at room temperature and potent fungal isolate was inoculated. The flask was incubated at 28±2^{o}C for five days (Puri et al., 2013).

Effect of moisture level

This was done by altering the level of moisture content in standard basal media using mineral media as moistening agent (Puri et al., 2013).The glucoamylase activity was determined by varying the moisture content in basal media at range of 1:1, 1:2, 1:3, 1:4 and 1:5 which were 5, 10, 15, 20 and 25ml of mineral medium /5g substrate.

Effect of IncubationTemperature

This was determined by incubating the inoculated standard basal media at temperature values ranging from 20°C, 27°C, 37°C, 45°C and 60°C (Sandhu et al., 2015).

Effect of Incubation Time

This was done by carrying out SSF for 1-5 days. At each day, the growth of fungi and enzyme activity was monitored (Sandhu et al., 2015).

Effect of pH

This was studied by altering pH of moistening agent at the range of pH 4.5, pH6, pH7.5, pH8and pH 9.5 using 1N HCl and 1N NaOH (Sandhu et al., 2015).

Enzyme extraction

Crude enzyme was extracted from fermented media by adding 0.1 M citrate phosphate buffer pH 5.5. The product was recovered from the substrate by shaking it for 30 minutes in shaking incubator (250rpm) with 0.1M citrate buffer (pH 5.5). Mixture was filtered through Whatman filter paper.The clear filtrate was then centrifuged at 5000 rpm for 20 minutes. The supernatant obtained was again filtered which was used as source of crude enzyme (Kheng and Omar, 2005).

Glucoamylase assay

Glucoamylase activity was determined according to the method given by (Cori, 1955; Puri et al., 2013)and reducing sugars liberated were determined by DNSA method (Miller, 1959).The amount of glucose released was determined by comparing the absorbance reading of the test enzyme at 540 nm with the standard graph of glucose.

RESULTS

In the present study, total seven fungal species were isolated from the soil sample, collected from flour mill effluents of Jabalpur (M.P.). The fungal isolates designated with codeAS*1 and AS*3were found to give maximum zone diameteras shown in Table 1.The positive results indicate capability of excessive amylase production showing larger zone in starch agar media.

Solid state fermentation using agro-industrial waste

The nature of solid substrate is important in solid state fermentation. The maximum zone forming fungal isolates AS*1 and AS*3were subjected tosolid state fermentation method for crude enzyme production. This was done using basal media which comprises of rice bran, wheat bran, wheat straw, rice husk collected from agricultural fields.In the present study, Rice bran was found to be most suitable for colonization of fungus as indicated by maximum enzyme activity and visual growth on the surface of substrate of fungal isolate AS*3 shown in Figure 1.Therefore in all the subsequent studies, AS*3 was used as potentfungal isolate and Rice bran was used as a substrate.

Study of Different Factor on SSF

In the present study, thedifferent cultural parameters like effect of moisture level, incubation temperature, incubation time and pH were also studied. This was done in order to optimize cultural conditions for exploitation of cheap agriculture residue rice bran by potent fungal isolate.

Effect of moisture level

The effect of moisture content was done by altering the level of moisture content in standard basal media using mineral media as moistening agent. The fungi are known to favour a moist environment for their growth and high moisture leads to particle agglomeration, gas transfer limitation and bacterial antagonism The various ratio of substrate (rice bran, rice husk, wheat bran and wheat straw) with mineral media used in the study were 1:1, 1:2, 1:3, 1:4 and 1:5. The maximum visible growth for glucoamylase synthesis was observed with rice bran with moisture level 1:4 as depicted in Table 2. The ratio 1:4 showed the glucoamylase activity of 8.6U/ml/min. This was followed by the ratio 1:3 with abundant visible growth and activity. The ratio 1:2 showed average growth and activity and the ratio 1:1 and 1:5 showed minimum growth for enzyme synthesis (Table 3).

Effect of Incubation Temperature

The incubation temperature is the most important physical parameter affecting SSF. The present study involves temperature variation at a range of 20°C, 27°C, 37°C, 45°C and 60°C keeping all other parameters constant. The maximum visible growth for glucoamylase synthesis was observed with rice bran at temperature 27°C as depicted in Table 4. The maximum glucoamylase activity was observed at 27°C with 3.12U/ml/min and the activity gradually decreases after 27°C as shown in the Table 5 and Figure 2. Therefore, in subsequent studies, fermentation was carried out at 27°C.

Effect of Incubation Time

The production of enzyme depends on the growth of fungi and incubation time is also an important factor in SSF. In the present study, the fungi was grown for regular interval for 5 days and at 3rd day, the maximum growth was found with rice bran depicted in Table 6. The glucoamylase activity of 3.04 U/ml/minwhich gradually decreased with incubation days as shown in Table 7 and Figure 3.The decrease in the activity may be due to the reduced consumption of nutrient materials.

Effect of pH

pH is one of the most important factor in SSF as fungi have their optimum pH range for maximum growth and enzyme synthesis. The study involves altering the pH of moistening agent at the range of pH 4.5, pH 6, pH 7.5, pH 8 and pH 9.5 using 1N HCl and 1N NaOH. Table 8 shows the maximum growth of fungus was observed at pH 6 with rice bran as substrate. The glucoamylase activity of 1.30 U/ml/min followed by pH 8 with activity 1.18 U/ml/min was observed as shown in Table 9 and Figure 4.

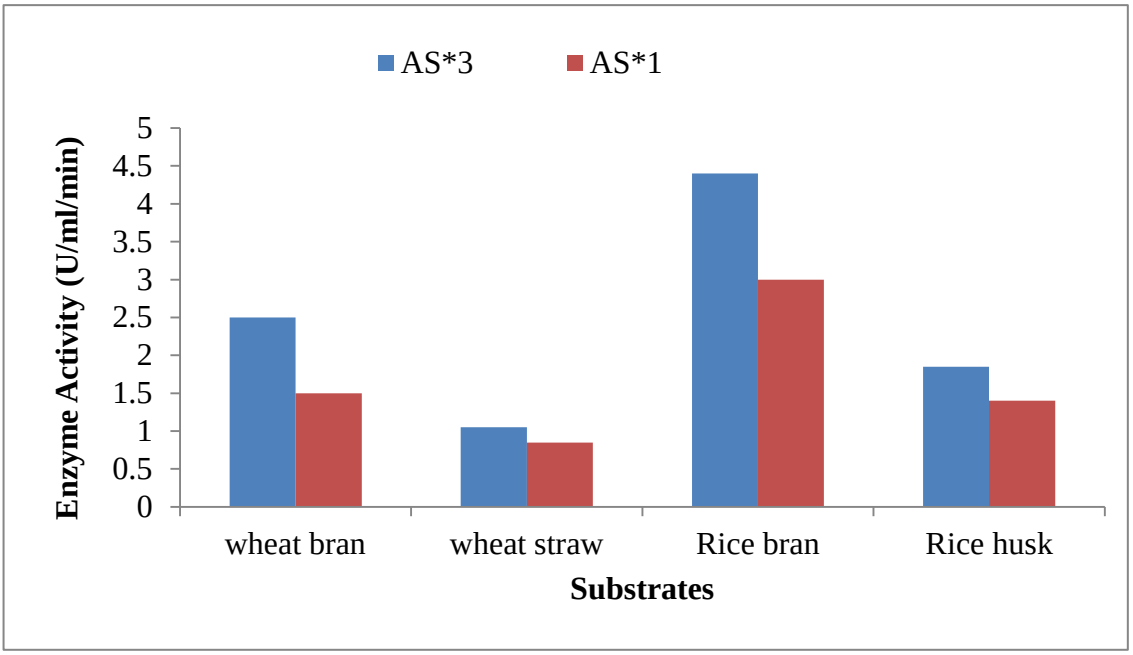

Figure 1: Effect of substrate on Enzyme activity of fungal isolates

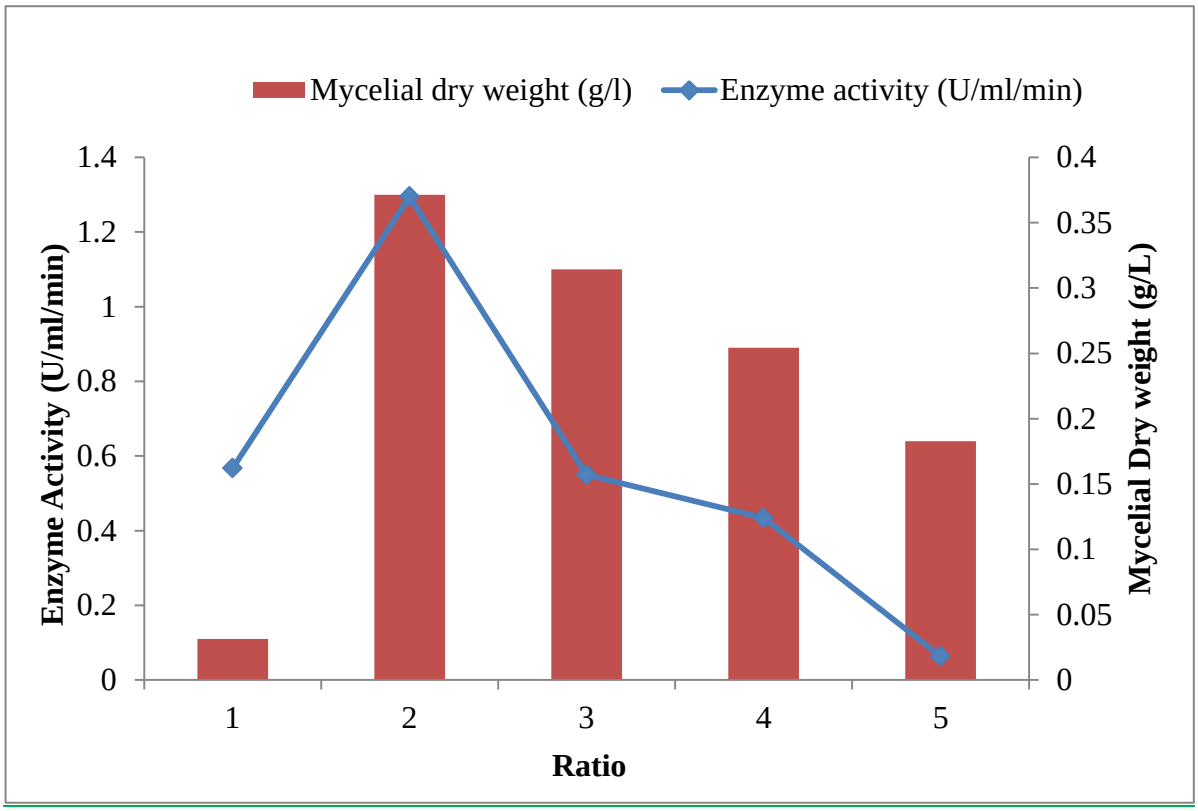

Figure 2: Effect of moisture level

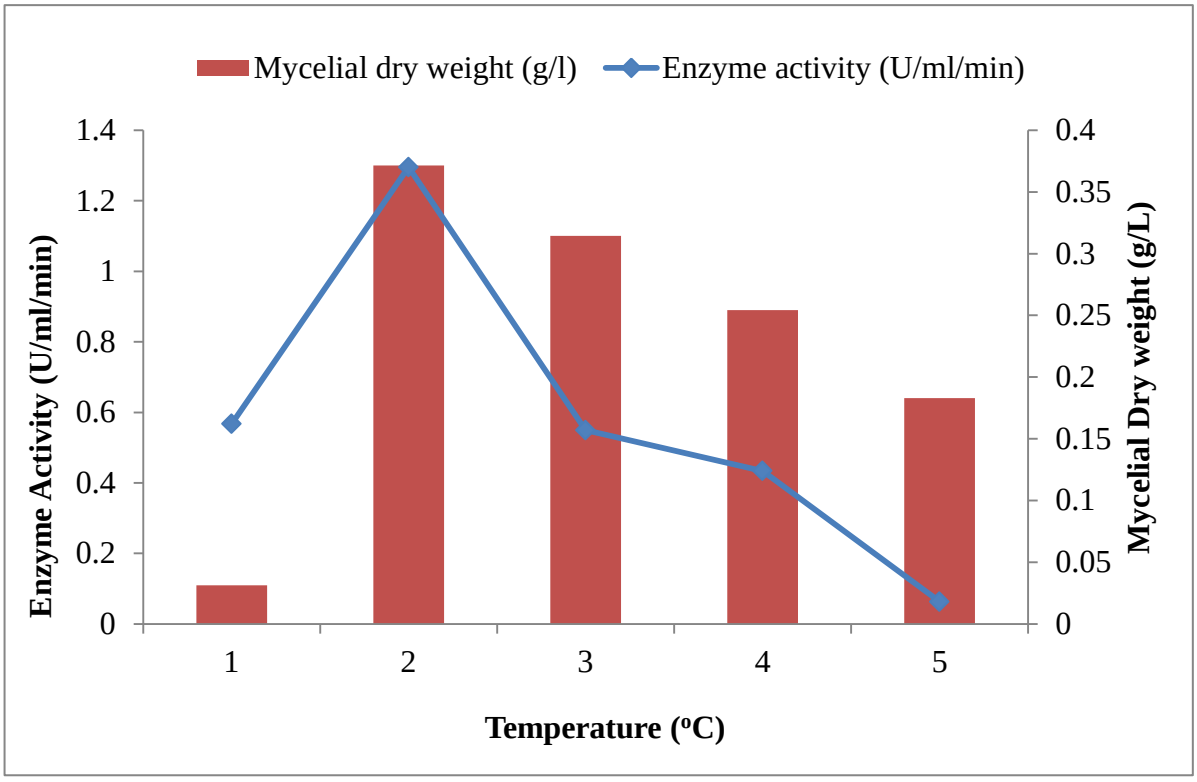

Figure 3: Effect of incubation temperature

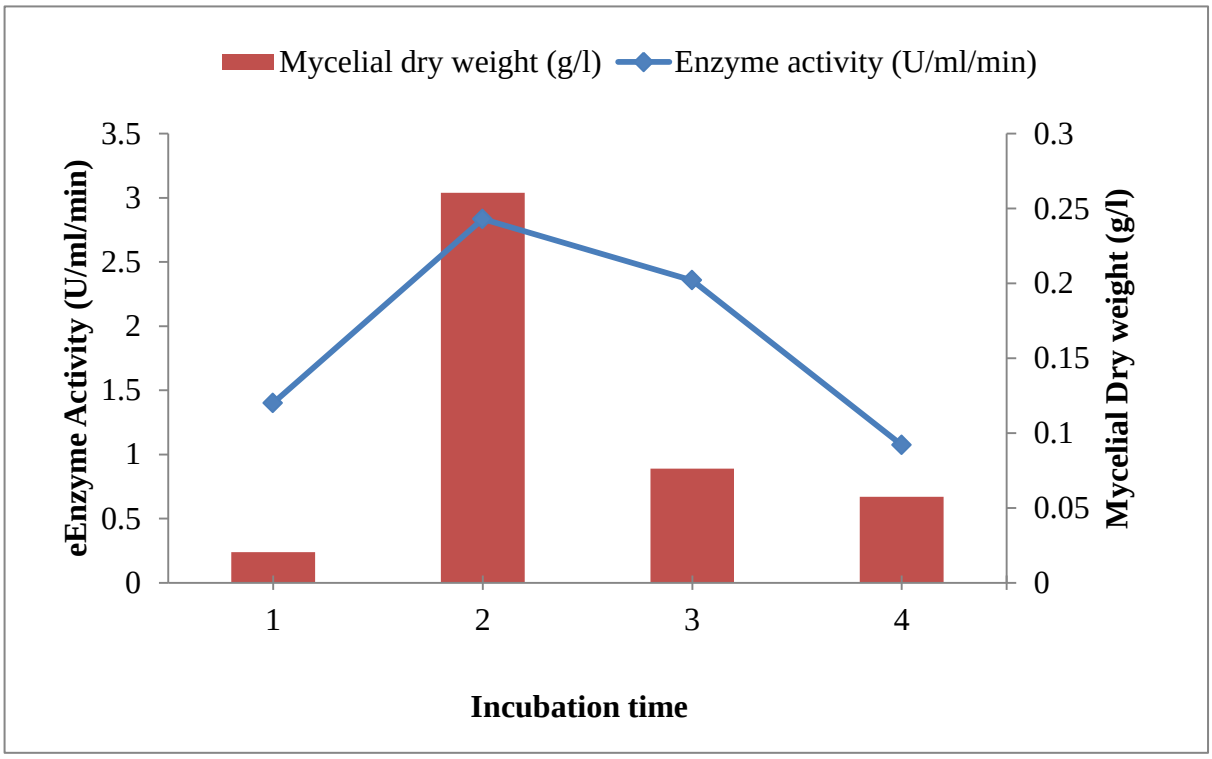

Figure 4: Effect of incubation time

Table 1: Starch hydrolysis test

S. No.	Strain Code	Zone diameter (mm)	Zone inference
1.	AS*1	22	Average (++)
2.	AS*2	13	Below average (+)
3.	AS*3	32	Abundant(+++)
4.	AS*4	15	Below average(+)
5.	AS*5	-	No zone(-)
6.	AS*6	-	No zone(-)
7.	AS*7	-	No zone(-)

#Growth variation indicated as (+) shows minimum growth, (++) shows average growth and (++++) shows abundant growth

Table 2: Growth variation of AS*3 on different Moisture level with substrates

S.No.	Ratio of Moisture level	Rice Bran	Rice husk	Wheat bran	Wheat straw
1.	1:1	++	+	+	+
2.	1:2	++	-	++	+
3.	1:3	+++	-	++	-
4.	1:4	++++	-	++	-
5.	1:5	++	-	+	-

#Growth variation indicated as (+) shows minimum growth, (++) shows average growth and (++++) shows abundant growth.

Table 3: Effect of moisture level

S. No.	Ratio (RB+MM)	Mycelial dry weight (g/l)	Enzyme activity (U/ml/min)
1.	1:1	0.214	5.44
2.	1:2	0.349	7.02
3.	1:3	0.397	8.44
4.	1:4	0.592	8.6
5.	1:5	0.405	6.93

#Enzyme activity calculated in terms of comparison of absorbance (at 540 nm) compared with the standard graph of glucose.

Table 4: Growth variation of AS*3 on different Incubation temperature with substrates

S. No.	Incubation Temperature (°C)	Rice Bran	Rice husk	Wheat bran	Wheat straw
1.	20	++	+	+	+
2.	27	++++	+	++	+
3.	37	++	-	+	-
4.	45	+	-	+	-
5.	60	-	-	+	-

Growth variation indicated by (+) shows minimum growth, (++) shows average growth and (++++) shows abundant growth.

Table 5: Effect of Incubation Temperature

S. No.	Incubation Temperature (°C)	Mycelial dry weight (g/l)	Enzyme activity (U/ml/min)
1.	20	0.092	0.52
2.	27	0.163	3.12
3.	37	0.148	1.74
4.	45	0.12	0.94
5.	60	0.10	0.31

#Enzyme activity calculated by DNSA Method in terms of absorbance (at 540 nm) compared with the standard graph of glucose

Table 6: Growth variation of AS*3 on different Incubation time with substrates

S. No.	Incubation Time (day)	Rice Bran	Rice husk	Wheat bran	Wheat straw
1.	2th	++	+	+	+
2.	3th	++++	+	++	+
3.	4th	++	-	+	-
4.	5th	+	-	+	-

Growth variation indicated as (+) shows minimum growth, (++) shows average growth and (++++) shows abundant growth.

Table 7: Effect of Incubation time

S. No.	Incubation Time (day)	Mycelial dry weight (g/l)	Enzyme activity (U/ml/min)
1.	2^{th}	0.12	0.24
2.	3^{th}	0.243	3.04
3.	4^{th}	0.202	0.89
4.	5^{th}	0.092	0.67

#Enzyme activity calculated by DNSA Method in terms of absorbance (at 540 nm) compared with the standard graph of glucose.

Table 8: Growth variation of AS*3 on different pH with substrates

S. No.	pH	Rice Bran	Rice husk	Wheat bran	Wheat straw
1.	4.5	++	+	+	+
2.	6	++++	+	++	+
3.	7.5	++	-	+	-
4.	8	+	-	+	-
5.	9.5	-	-	+	-

Growth variation indicated by (+) shows minimum growth, (++) shows average growth and (++++) shows abundant growth.

Table 9: Effect of pH

S. No.	pH	Mycelial dry weight (g/l)	Enzyme activity (U/ml/min)
1.	4.5	0.162	0.11
2.	6	0.37	1.3
3.	7.5	0.157	1.1
4.	8	0.124	0.89
5.	9.5	0.018	0.64

#Enzyme activity calculated by DNSA Method in terms of absorbance (at 540 nm) compared with the standard graph of glucose

DISCUSSION

Starch is one of the most important naturally occurring glucose polymers used as a starting material for production of a variety of components of commercial importance. Glucoamylase is one of the widely used biocatalysts in food industry. The use of agricultural residues for glucoamylase production offers alternative use of cheap residues which is potential strategy for achieving economy in alcohol manufacturing industries. The present study shows isolation of total seven fungal species from soil sample collected from flour mill effluents of Jabalpur (M.P.). Similarly, Mishra et al. (2010) studied isolation of filamentous fungi from soil of different regions of Rajasthan state. The starch hydrolyzing capability of the fungal isolates was evaluated by starch hydrolysis test. Similarly, Abu et al. (2005) reported screening of glucoamylase by starch hydrolysis test.

Various soil fungi are employed in starch processing by glucoamylase. The main aim of the present study was production of glucoamylase by solid state fermentation using cheap agricultural residues. Similarly, Lam et al.(2013) reported use of solid state fermentation for the synthesis of glucoamylase. The growth of fungus and production of enzymes and secondary metabolites are sensitive to temperature, pH, incubation time and incubation temperature. Therefore, these cultural parameters were also optimized for the maximum production of glucoamylase in the present study. In the present study, solid state fermentation was carried with different substrates (rice bran, rice husk, wheat bran and wheat straw),at temperatures (20°C, 27°C, 37°C, 45°C and 60°C) , pH (4.5, 6, 7.5, 8 and 9.5), incubation time (regular interval for 5 days). Similarly, Puri et al. (2013) shows the effect of moisture content by using mineral media as a moistening agent, incubation temperature, incubation time and pH. Similar observation was made by Parbat et al.(2011) and showed production of Glucoamylase by *Aspergillus oryzae* under solid state fermentation using agro-industrial products where the maximum yield was achieved with optimized process parameters such as incubation period (120 hours), pH 5.0 and fermentation temperature(60°C).The results reveals rice bran as the best agriculture residue with moisture level at the ratio of 1:4 at temperature 27°C, pH 6 and 3days of incubation for optimum production of glucoamylase.

CONCLUSION

The demand of glucoamylase is increasing continuously with increase in its application spectrum. Thus, significant interests exist in research on the amylolytic enzymes as the conventional production of ethanol from starch. The present study shows the potential of agro-industrial residues as an economical method for glucoamylase production. The native isolate of soil was exploited by SSF of the cheap readily available agro-products. Out of different agricultural residues tested, maximum glucoamylase activity was found with rice bran. The study of different cultural amendments was done and from the findings it can be concluded that the rice bran: mineral media (1:4), temperature 27°C, pH 6 and 3days of incubation is suitable for optimal glucoamylase synthesis. The study explores cost effective usage of agro- based residues by soil mycotic flora thereby favoring its use in fermentation and food industries. However, further research is needed in this direction for the study the effect of other cultural parameters like substrate concentration, nitrogen source, carbon source, surfactants, metal ions, vitamins, inoculums size etc.

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