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INDUSTRIAL ENZYME PRODUCTION BY *MUCOR GEOPHILLUS* IBGE 05

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ABSTRACT

Optimization parameters in submerged fermentation were studied for the production of *α*-amylase from *Mucor geophillus* IBGE 05 using agricultural wastes (sunflower waste, cotton stalk and rice husk) as well as agro industrial wastes (date syrup and molasses) as sources of carbon. Optimal conditions for the production of *α*-amylase (4.87 U/mL) by *Mucor geophillus* IBGE 05 were observed when the strain was grown on culture medium M1 containing yeast extract as a source of nitrogen, molasses as a source of carbon after 48 h of incubation at 35° C, initial pH 6.5, inoculum size of $5x10^6$ conidia in 50 mL of culture medium and agitation rate of 150 rpm. The strain was thermostable (up to 60° C) and pH (up to 9.0) so it has a potential to be used in industries for enzyme production.

Key words: Optimization, *Mucor geophillus*, date syrup, alpha amylase

INTRDUCTION

Alpha-amylase (Enzyme Commission No. is 3.2.1.1) is an extracellular enzyme, which splits *α*-1, 4- glycosidic bonds of starch and produces glucose, maltose and alpha limit dextrin (Leman *et al.*, 2009).The substrate of amylase is starch, which is a polysaccharide and composed of two types of polymers amylose and amylopectin. Starch is composed of 20-25 % amylase, which is a linear chain of glucose units joined by *α*-1, 4- glycosidic bonds and about 75-80 % amylopectin, which is branched macro molecule of glucose in which 1, 6- glycosidic bonds are also present (Sundarram and Murthy, 2014).

Amylases are the most widely used commercial enzymes whose range of application has broadened in numerous areas such as food and medicinal and clinical and analytical chemistry. They are used in starch hydrolysis, in pharmaceutical, food, baking, and brewing, paper, detergent and textile industries. These are essential enzymes used in starch treating activities for hydrolysis of polysaccharides such as starch into simple sugar components (Sundarram and Murthy, 2014).

In this work optimization parameters in submerged fermentation were studied for the production of *α*-amylase from *Mucor geophillus* IBGE 05 using agricultural wastes (sunflower waste, cotton stalk and rice husk) as well as agro industrial wastes (date syrup and molasses) as sources of carbon.

MATERILS AND METHODS

Strains

Strain of *Mucor geophillus* IBGE 05 was obtained from the Institute of Biotechnology and Genetic Engineering, University of Sindh Jamshoro and cultures were maintained as followed by Dahot (1986). In the present study slants of 4 days old were used for inoculation.

Conidia count

Number of conidia of each fungus was counted by haemocytometer. Spore suspension was maintained about $4x10^6$ conidia/mL and they were added to 50 mL of fermentation media in 250 mL flask.

Hydrolysis of agriculture waste

Each agricultural waste (cotton stalk, sunflower waste and rice husk) was treated as reported earlier (Ahmed *et al*., 2011).

α-Amylase Activity (Bernfeld, 1955)

Enzyme sample of 1.0 mL was added in 1.0 mL of 1 % (w/v) soluble starch in 50 mM sodium phosphate buffer at pH 7.0 and then incubated for 3 min. at 50° C then 1.0 mL DNS reagent was added and boiled for 15

min. and cooled at room temperature, later on absorbance was noted at 540 nm.

One unit of α-amylase is the amount of enzyme that will release 1 mg of reducing sugar in 3 min at 50° C and pH 7.0.

Optimization of Enzyme Production Parameters

All experiments were done in such a way that the parameter optimized in one experiment was fixed in the other experiments for the production of enzyme.

Culture media

First of all the most suitable culture medium was determined. For optimization of α-Amylase production following culture media were used having composition (g/L).

M1: Dextrose 10, Peptone 5, Epsom salt 5, KH_2 PO_4 5, Common salt 2.5, ferrous sulphate hepta hydrate 0.01, $ZnSO_4.7H_2O$ 0.002, $MnSO_4.H_2O$ 0.001 and thiamine hydrochloride 0.001 (Burrel *et al.,* 1966).
M2: soluble starch 20, $NH_4NO_3$10, KH_2PO_4, 14, KCl, 0.5, Epsom salt 0.1, $FeSO_4.7H_2O$, 0.01 (Matthias, 2013).
M3: NaCl 0.8, KCl 0.8, $CaCl_2$ 0.1, Na_2HPO_4 2.0, $MgSO_4$0.2, $FeSO_4$ 0.1, 8.0 Glucose, NH_4 Cl 2.0 (Khan & Yadav, 2011).
M4: Zn $SO_4.7H_2O$ 0.062, $FeSO_4$ 0.068, copper sulphate pent hydrate 0.0001 and wheat bran 100 (Hayashida & Teramoto, 1986).

Incubation time period

After the determination of the most suitable culture medium, optimum incubation time period was determined. It was done by growing the strain on M1 (which were observed the most suitable for all fungi under investigation) at various time periods from 24-240 h.

Carbon sources

After the optimization of incubation time the most suitable carbon source was determined. It was done by replacing the glucose (control) of culture medium (M1) by various wastes including sunflower waste, cotton stalk, rice husk, which were hydrolyzed by 0.3 *N* H_2SO_4 and 0.6 *N* H_2SO_4. Date syrup and molasses were used 0.5 % and 1 % in place of glucose (control).

Nitrogen sources

After the determination of the most suitable carbon source various nitrogen sources were checked for optimum production of enzymes. It was done by replacing peptone of culture medium (M1) by corn steep liquor, casein, potassium nitrate, albumin, ammonium sulphate, urea and yeast extract.

Incubation temperature

The most suitable culture medium M1 (with the most suitable carbon and nitrogen source) was tested on varying temperature from 20-70° C to determine the most suitable incubation temperature for the production of enzymes.

Initial pH of medium

The initial pH of a medium has an effect on growth and productivity of microorganism. A range of pH from 4.0-9.0 was checked for optimum enzymes production.

Inoculum size

Productivity was also checked in terms of number of conidia in 50 mL of optimized culture medium in order to obtain the optimized inoculum size of culture medium. The number of conidia was counted by haemocytometer.

Agitation rate

Effect of agitation rate was also checked for optimization at 50, 100, 150, 200, 250 and 300 rev/min in orbital shaking incubator.

RESULTS AND DICUSSIONS

Effect of culture media

Effects of various culture media on *α*-amylase production by *M. geophillus* IBGE 05 after 24 h, at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are presented in Fig. 1. The strain was grown on four different culture media *i.e.* M1, M2, M3 and M4. It was capable of growing well on all types of culture media but production of α-amylase was maximum (1.02 U/mL) on culture medium M1, which was selected for the next study of α-amylase production.

Effect of incubation time period

The effects of incubation time periods on *α*-amylase production by *M. geophillus* IBGE 05 in M1 at temperature 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are plotted in Fig. 2. Activity of *α*-Amylase was measured at regular interval of 24 h and it was found that the maximum activity (1.73U/mL) was observed after 48 h of incubation. On prolonged incubation enzyme activity was decreased, which might be due to denaturing of enzyme or synthesis of inhibiting metabolite (Mamma *et al*., 2008). Khan and Yadav (2011) reported incubation time period of 48 h for *α*-amylase production by *Aspergillus niger*.

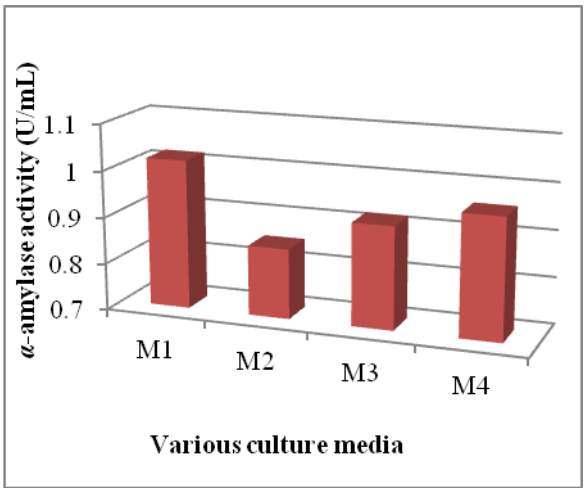

Fig. 1. Effects of various culture media on *α*-amylase production by *M. geophillus* after 24 h, at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

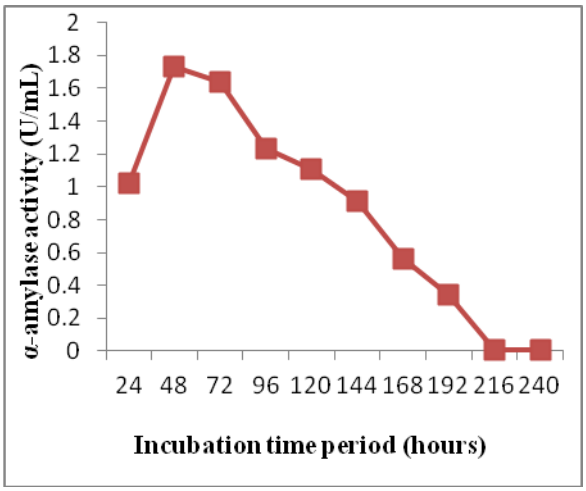

Fig. 2. Effects of incubation time periods on *α*-amylase production by *M. geophillus* in M1 at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

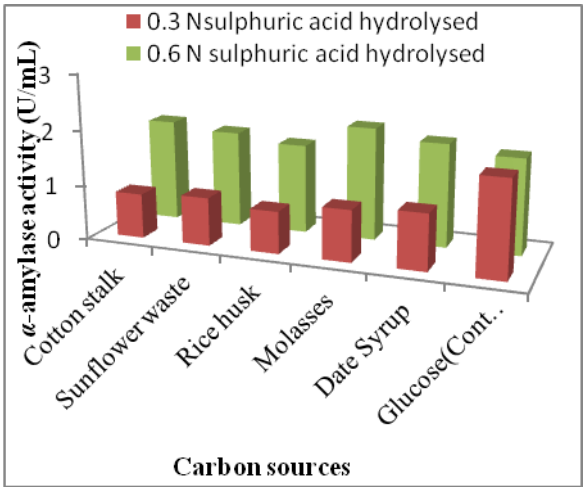

Fig. 3. Effects of various carbon sources on production *α*-amylase by *M. geophillus* after 48 h in M1 at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

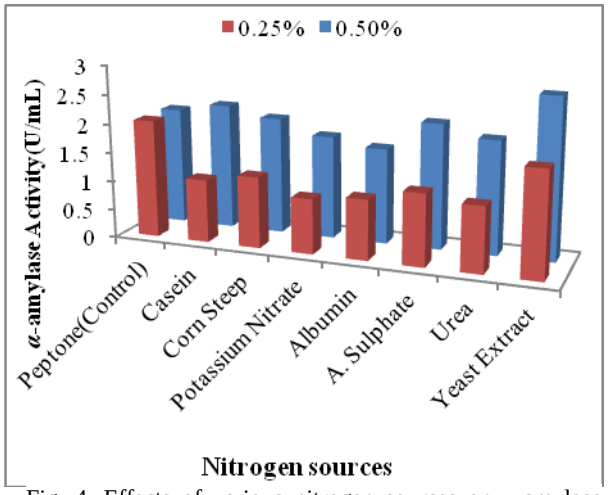

Fig. 4. Effects of various nitrogen sources on *α*-amylase production by *M. geophillus* after 48 h in M1 containing molasses as carbon source at t 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

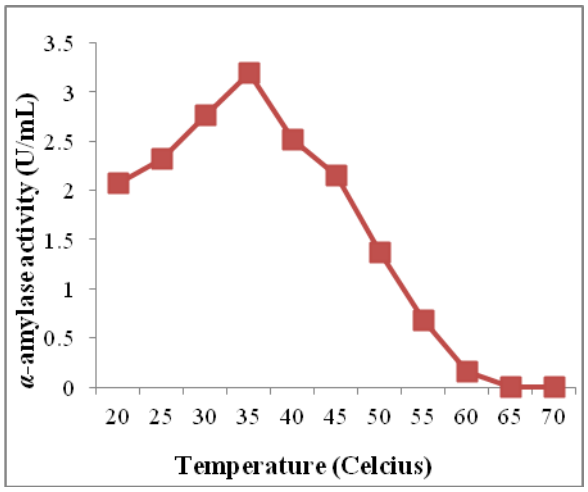

Fig. 5. Effects of incubation temperatures on *α*-amylase production by *M. geophillus* after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, at initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

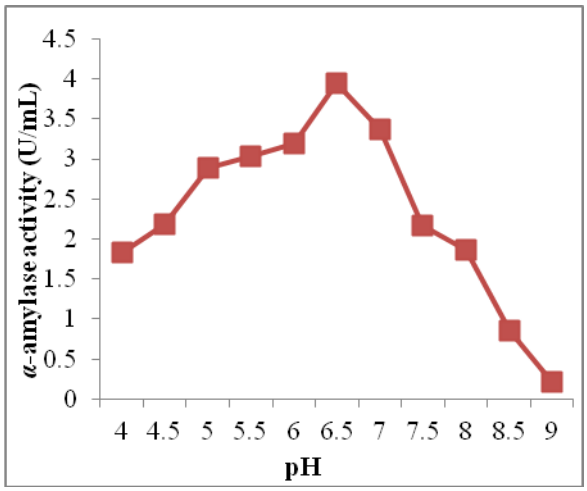

Fig. 6. Effects of initial pH of fermentation medium on *α*-amylase production by *M. geophillus* after 48 h in M1 containing molasses as carbon source, yeast extract nitrogen source, at 35º C, inoculum size $4x10^6$ conidia and agitation rate 50 rev/min.

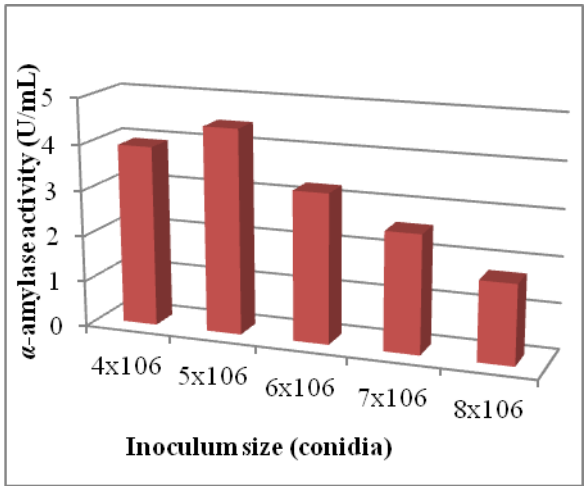

Fig. 7. Effects of inoculum sizes on *α*-amylase production by *M. geophillus* after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, at 35º C, initial pH 6.5 and agitation rate 50 rev/min.

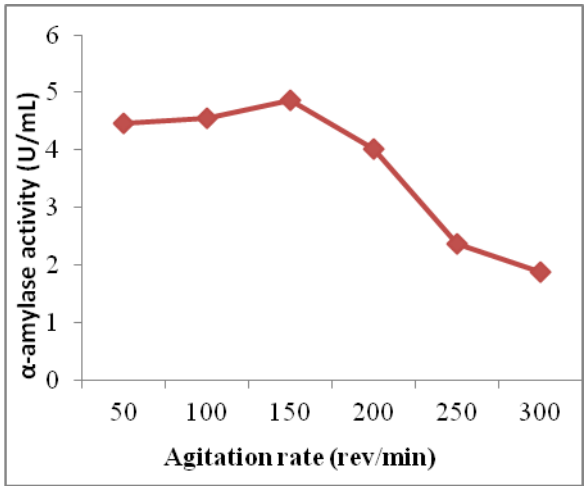

Fig. 8. Effects of agitation rates on *α*-amylase production by *M. geophillus* after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, at 35º C, initial pH 6.5 and inoculum size $5x10^6$ conidia.

Effect of carbon sources

The effects of various carbon sources on production *α*-amylase by *M. geophillus* IBGE 05 after 48 h in M1 at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are shown in Fig. 3. It was observed that *α*-amylase activities were lower in case of 0.3*N* sulphuric acid hydrolysed agriculture waste(0.81, 0.87 and 0.76 U/mL for cotton stalk, sunflower waste and rice husk respectively) and 0.5 % of molasses and date syrup (0.93 and 1.01 U/mL respectively). Activities of *α*-amylase were higher than control, glucose (1.02U/mL) when 0.6*N* sulphuric acid hydrolysed agriculture waste (1.86, 1.75 and 1.62 U/mL for cotton stalk, sunflower waste and rice husk respectively) and 1 % of molasses (2.04 U/mL) and date syrup (1.87 U/mL) were used. Various carbon sources reported for *α*-amylase prodction by different researchers (Archana and Satyanarayana, 2011; Matthias, 2013;

Saleem and Ebrahim, 2014; Singh *et al*., 2014).

Effect of nitrogen sources

The effects of various nitrogen sources on *α*-amylase production by *M. geophillus* IBGE 05 after 48 h in M1 containing molasses as carbon source at 30° C, initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are given (Fig. 4). The strain showed the capability of utilizing well all types of nitrogen sources but yeast extract was found the best (1.82 U/mL in 0.25 % and 2.76 U/mL in 0.50 %). Various nitrogen sources reported for *α*-amylase prodction by different researchers (Archana and Satyanarayana, 2011; Matthias, 2013; Saleem and Ebrahim, 2014; Singh *et al*., 2014).

Effect of temperature

The effects of incubation temperatures on *α*-amylase production by *M. geophillus* IBGE 05 after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, at initial pH 6.0, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are exhibited (Fig. 5). The fermentation medium was incubated at a range of temperatures 20-70° C. Activity of *α*-amylase was the highest (3.19 U/mL) about 35° C. The strain showed thermo stability up to 60° C (0.16 U/mL). Singh *et al*. (2014) also reported 35° C for *α*-amylase production by *Aspergillus fumigatus.*

Effect of initial pH

The effects of initial pH of fermentation medium on *α*-amylase production by *M. geophillus* IBGE 05 after 48 h in M1 containing molasses as carbon source, yeast extract nitrogen source, temperature 35° C, inoculum size $4x10^6$ conidia and agitation rate 50 rpm are presented (Fig. 6). The range of pH (4.0 to 9.0) was studied and found that initial pH of 6.5 was the best for maximum enzyme production (3.94 U/mL). Various initial pH of fermentation medium have been reported for *α*-amylase prodction by different researchers (Archana and Satyanarayana, 2011; Matthias, 2013; Saleem and Ebrahim, 2014; Singh *et al*., 2014).

Effect of inoculum size

The effects of inoculum sizes on *α*-amylase production by *M. geophillus* IBGE 05 after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, at 35° C, initial pH 6.5 and agitation rate 50 rpm are plotted (Fig. 7). Flasks were added with $4x10^6$-$8x10^6$ conidia and maximum *α*-amylase activity (4.47 U/mL) was observed when $5x10^6$ conidia were added to the medium. Researchers have used various inoculum sizes (Archana and Satyanarayana 2011). Large inoculum size caused overgrowth and nutritional imbalance resulting less production of enzyme (Dahot, 1986; Mamma *et al.,* 2008; Archana and Satyanarayana, 2011).

Effect of agitation rate

The effects of agitation rates on *α*-amylase production by *M. geophillus* IBGE 05 after 48 h in M1 containing molasses as carbon source, yeast extract as nitrogen source, temperature 35° C, at initial pH 6.5 and inoculum size $5x10^6$ conidia are given (Fig. 8). The fermentation medium was agitated at 50, 100, 150, 200, 250 and 300 rpm. Activity of *α*-amylase was maximum (4.87 U/mL) at 150 rpm. Researchers have reported various agitation rates (100-200 rpm) for enzymes production by different microorganisms (Dahot, 1986; Mamma *et al.,* 2008; Archana and Satyanarayana, 2011).

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