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Full Length Research Paper

Parametric optimization of extracellular α-amylase recovery from the fermented bran of *Aspergillus awamori* MTCC 9997

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A newly isolated *Aspergillus awamori* MTCC 9997 strain from spoiled cooked rice was cultured under solid state using cassava peel powder to produce α-amylase. The effect of extraction medium, solid to solvent ratio, extraction time, temperature and physical state on α-amylase recovery was examined. Among various organic, inorganic solvents and buffer solutions tested, maximum extraction of α-amylase was achieved when phosphate buffer of pH 7.0 was added. A solid to solvent ratio (cassava peel : phosphate buffer) of 1:10 (w/v) resulted in maximum yield of α-amylase. The optimum extraction time was 30 min. At 30°C, α-amylase recovery was found to be the maximum. An appreciable amount of α-amylase was recovered under agitated conditions of the fermented biomass at 150 rpm compared with the quantum recovered under stationary conditions.

Key words: α-Amylase, *Aspergillus awamori*, solid-state fermentation, extraction.

INTRODUCTION

Microbial α-amylases are highly demanded industrial enzymes. They are employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Abu et al., 2005). α-Amylase (endo-1,4-α-D-Glucan glucanohydrolase, E.C. 3.2.1.1) randomly cleaves the 1,4-α-D-glycosidic linkages between the adjacent glucose units in linear amylose chain in starch and related oligosaccharides and polysaccharides (MacGregor et al., 2001).

Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, baking, brewing, textile to paper industries (Rosell et al., 2001; Habibi et al., 2006). Amylases are used for the pre-treatment of animal feed to improve digestibility, in pharmaceuticals and for sewage treatments (Chi et al., 2001; Demirkan et al., 2005). Amylases accounts approximately about 25% of the enzyme market and almost completely replaced chemical hydrolysis of starch in starch processing industry (Pandey et al., 2000).

α-Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Gupta et al., 2003). Demand for microbial α-amylases has increased due to their speci-ficity of reaction, mild conditions required for reaction and less energy consumption than the conventional chemical methods (Thippeswamy et al., 2006). Fungal α-amylases are produced by different fermentation techniques. However, filamentous fungi are best adopted for solid-state fermentation (SSF).

The potential of SSF lies in bringing the cultivated microorganism in close vicinity of substrate and achieving the highest substrate concentration for the fermentation. SSF resembles the natural habitat of microorganism and is, therefore, the preferred choice for microorganisms to grow and produce useful value added products. Sub-merged fermentation can be considered as a violation to their natural habitat, especially of fungi. Not even marine microorganisms prefer swimming in free water, since

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more than 98% of isolates from marine environment have been obtained from the underwater surfaces of solid substrates, found in marine habitats (Holker et al., 2004). In solid-state fermentation the products are formed at or near the surfaces of the solid materials with low moisture content (Selvakumar and Pandey, 1999). So it is necessary to select a solvent for leaching out the product from the fermented mass.

This paper reports the optimization of extraction parameters for α-amylase recovery from *Aspergillus awamori.*

MATERIALS AND METHODS

Microorganism

The organism used was *Aspergillus awamori* MTCC 9997, a strain isolated from spoiled cooked rice. It was maintained on rose bengal chloramphenicol agar slants. The culture was subcultured periodically after 30 days and stored at 4ºC.

Fermentation

Fermentation was carried out in 250 ml Erlenmeyer flasks containing 5 g of wheat bran. The substrate was moistened with 10 ml of distilled water, autoclaved (15 lb) at 121ºC for 15 min and cooled. The flasks were inoculated with 1% (v/w) inoculum of *Aspergillus awamori* and incubated at 28ºC for 96 h.

Enzyme extraction

The enzyme from the fermented bran was extracted twice with 50 ml of solvent solution. The contents were mixed by shaking for 15 min at 28ºC on a rotary shaker at 150 rpm. The slurry was squeezed through a damp cheese cloth. The extracts were pooled and centrifuged at 4ºC for 15 min at 5000 rpm to separate small wheat bran particles, cells and spores. The brown clear supernatant was used as the source of α-amylase.

Effect of extraction medium

The extraction of α-amylase from the fermented fungal biomass was carried out with distilled water, tap water, inorganic salt solutions (Potassium chloride, calcium chloride and sodium chloride) at a concentration of 0.1%, organic solvents (glycerol, methanol, ethanol and acetone) at a concentration of 1% and buffers (0.2 M acetate buffer pH 5 and 0.2 M phosphate buffer pH 7). Extraction was carried out for 15 min and α-amylase activity was determined. The most suitable extraction medium determined was used for subsequent experiments.

Effect of solid to solvent ratio on the extraction of α-amylase

To investigate the effect of solid to solvent ratio on the extraction of α-amylase, different volumes of phosphate buffer (optimum extraction medium) in the ratio 1:1, 1:5, 1:10, 1:15, 1:20 and 1:25 w/v were used. α-Amylase activity was assayed. The optimized solid to solvent ratio was used for further studies.

Effect of extraction time on α-amylase recovery

The incubation time for extraction of α-amylase was optimized by incubating the fermented substrate with phosphate buffer (1:10 w/v) for different time intervals. The time period was varied from 15, 30, 45, 60 and 75 min and α-amylase activity was assayed after each incubation time.

Effect of extraction temperature

To study the effect of temperature on the extraction process, the temperature was varied from 20 to 60ºC at 10ºC intervals.

Effect of physical state

Two different extraction conditions were studied, stationary and agitation (150 rpm).

Enzyme assay

α-Amylase activity was determined by the method described by Bernfeld (1955) using 3,5-dinitrosalicylic acid. The absorbance was measured at 540 nm. One unit of α-amylase activity is defined as the number of µmol of maltose liberated by one ml of enzyme solution per minute. α-Amylase activity is expressed per gram of dry substrate (gds). All values are averages of three determinations.

RESULTS AND DISCUSSION

Effect of extraction medium on α- amylase recovery

The extraction efficiency is critical to the recovery of enzyme from the solid fermented biomass as it is carried out in the absence of free liquid and therefore selection of a suitable solvent is necessary. Organic, inorganic solvents and buffers besides tap and distilled water were used in this study.

From the result, it is clear that among the solvents used, phosphate buffer was found to be best for the extraction of α-amylase from the fermented solids of *Aspergillus awamori* (35.02 U/gds) which indicates that buffers are more effective as soaking solvents (Figure 1). Gangadharan et al. (2006) reported acetate buffer to be the best solvent for α-amylase recovery from *Bacillus amyloliquefaciens*. However, Palit and Banerjee (2001) reported glycerol as the best extraction medium for α-amylase from *Bacillus circulans* GRS 313.

Effect of solid to solvent ratio on α- amylase recovery

In solid-state fermentation system, free flowing solvent is very much limited. Thus adequate amount of solvent is required to leach out the enzyme present. It was observed that 5 g of fermented bran with 50 ml solvent (solid to solvent ratio of 1:10) was optimum for α-amaylase extraction from *Aspergillus awamori* (Table 1). Above or below this ratio, there was decline in enzyme yield. Decrease in α-amylase yield was noticed when

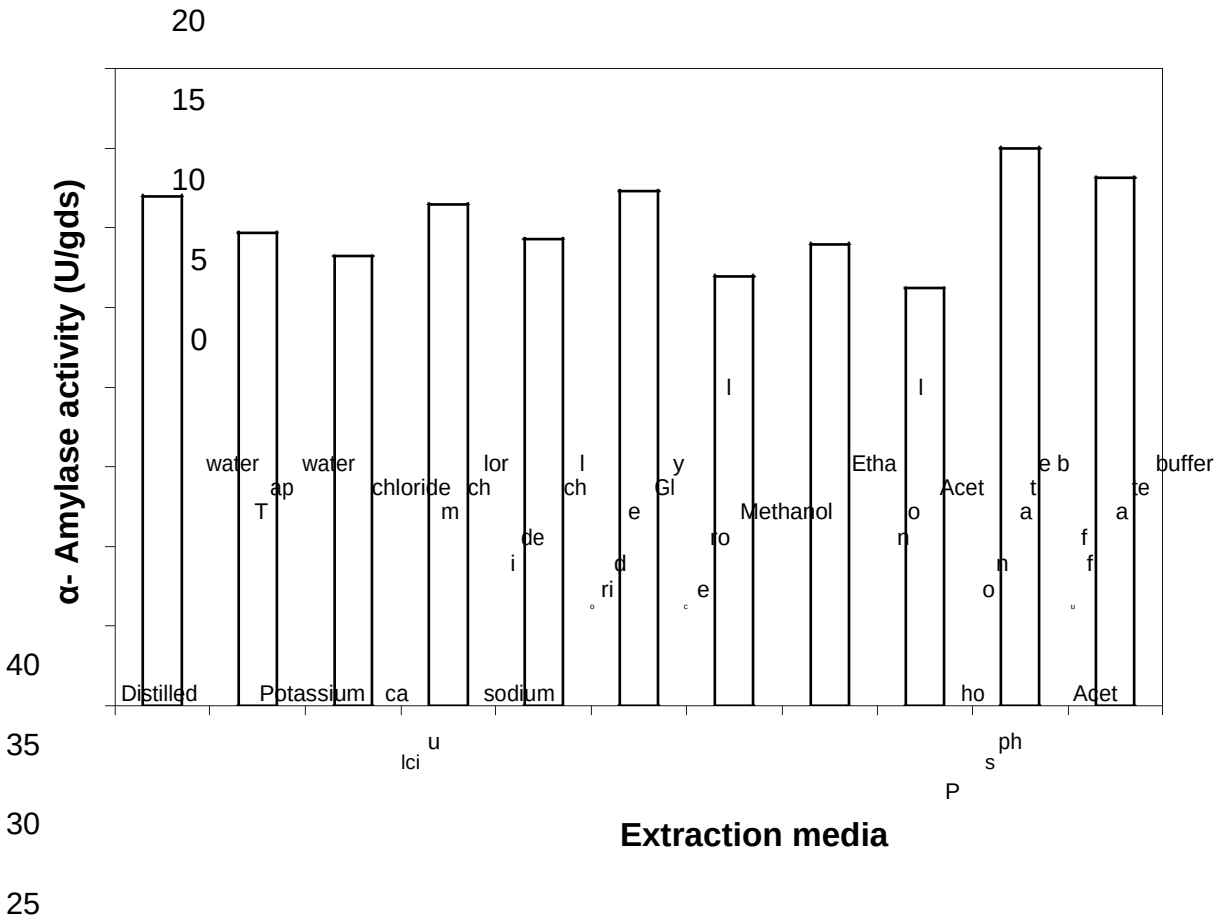

Figure 1. Effect of extraction medium on α-amylase recovery.

Table 1. Effect of solid to solvent ratio on α- amylase recovery.

Solid to solvent ratio (w/v)	α- Amylase activity (U/gds)
1:1	26.34±0.59
1:5	30.39±1.20
1:10	47.30±1.86
1:15	35.64±3.38
1:20	32.08±3.75
1:25	22.51±0.88

U/gds – Units per gram of dry substrate. Values are mean of three replicates ± SD.

lower volume of solvent was used. This might be due to insufficient solvent volume to penetrate the solid fermented mass. Excessively large volume of extractant would also yield too dilute enzyme solutions to be profitably utilized (Palit and Banerjee, 2001).

Similar to the findings of the present study, Ahmed (2008) reported 1:10 as the optimum solid to solvent ratio for the extraction of levan sucrase from *Bacillus megaterium*. However, Palit and Banerjee (2001) recorded an optimum solid to solvent ratio of 1:3 for amylase recovery from *Bacillus circulans* GRS 313.

Effect of extraction time on α- amylase recovery

Keeping the solid to solvent ratio at optimum level,

Table 2. Effect of extraction time on α- amylase recovery.

Time (min.)	α-Amylase activity (U/gds)
15	30.15±0.97
30	39.27±1.55
45	38.88±1.79
60	35.83±1.37
75	34.68±1.14

U/gds – Units per gram of dry substrate. Values are mean of three replicates ± SD.

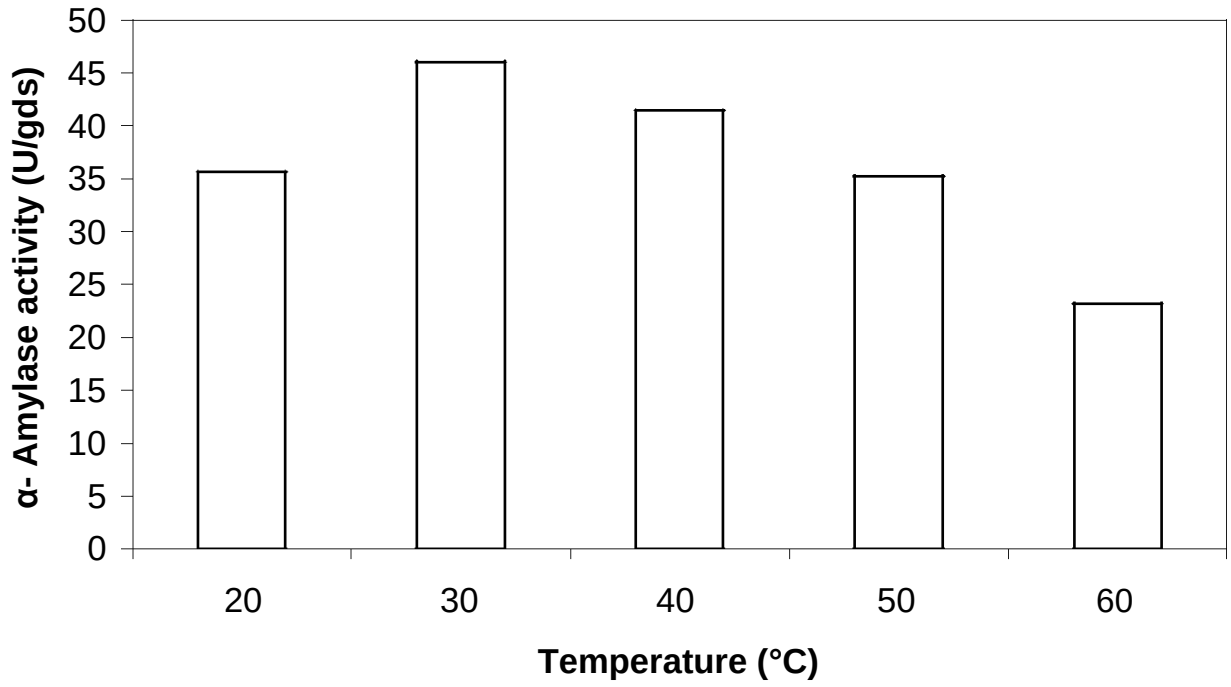

Figure 2. Effect of temperature on α- amylase recovery.

extraction time was optimized for maximum enzyme recovery from the fermented wheat bran. It was observed from Table 2, that 30 min soaking was the optimum time for α-amylase extraction and beyond that it did not have any additional effect on enzyme extraction indicating that 30 min is the minimum time required for the total penetration of solvent through the fermented biomass, whereas 15 min period is not enough for total solubilization of α-amylase.

Castilho et al. (2000) reported 30 min as the optimum time for the extraction of pectinases from *Aspergillus niger*. Ahmed (2008) investigated the effect of soaking time on levansucrase extraction from *Bacillus megaterium* and found that 90 min was optimum. On the other side, Palit and Banerjee (2001) recorded 150 min as the optimum extraction time for the recovery of amylase from the fermented bran of *Bacillus circulans* GRS313.

Effect of temperature on α- amylase recovery

It is clear from Figure 2 that 30°C was the optimum temperature for leaching of the enzyme from the fermented bran of *Aspergillus awamori*. However, at higher temperatures the enzyme yield was less. This may be due to denaturation of enzymes at higher temperatures.

Effect of physical state on α-amylase recovery

Agitation of the fermented biomass at 150 rpm with phosphate buffer at 30°C gave appreciable amount of α-amylase compared with stationary condition (Figure 3). This might be due to the fact that on agitation fermented bran gets distributed uniformly in the continuous phase of solvent, reducing concentration polarization (Tunga et al.,

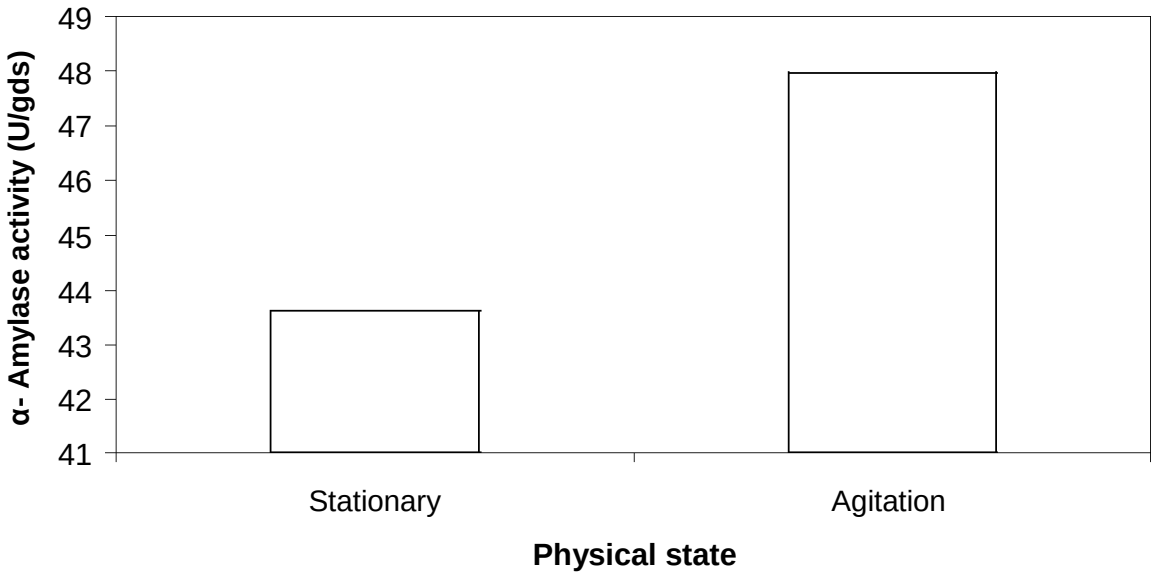

Figure 3. Effect of physical state on α-amylase recovery.

1999).

In conclusion, the parametric optimization of α-amylase recovery from the fermented bran of *Aspergillus awamori* shows that phosphate buffer at pH 7 with a solid to solvent ratio of 1:10 was best. Maximum yield was achieved when agitated for 30 min at 30°C.

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