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Commercial production of alpha amylase enzyme for potential use in the textile industries in Bangladesh

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Abstract

In Bangladesh textile and apparel industry is in the leading position in industrial structure. It contributes 12% of the country's GDP and also accounts for almost 78% of total exports, making it countries leading foreign exchange earner. Alpha amylase is an enzyme used in textile industry for smoothening the fabric. It is also used in many washing powder, food and paper industries. But yet we do not produce this enzyme in Bangladesh though its production and purification technique is rather simple. Each year we spend a huge amount of foreign currency to import it. In this study we scaled down the production cost of alpha amylase in a pilot project. *Aspergillus niger* was used in this project for alpha amylase production. *A. niger* was cultured in PDB (Potato Dextrose Broth) medium for seed production. Wheat bran was used as substrate for fermentation. Substrates were pre-treated with 1% NaOH for hydrolysis and washed with dH_2O until neutral pH obtained. Substrates were then dried overnight using oven. MSM (Minimum Salt Medium) medium with some modifications was used for fermentation. Fermentation was carried out at 28°C at a pH of 6.2. Extraction of the enzyme was carried out by centrifugation. CMC and DNS assay showed good performance of the extracted enzyme. The calculated production cost of our enzyme is 57.92 taka per liter which is cheaper than imported ones (~450 taka per liter). Therefore, we strongly recommend that entrepreneur should be contacted to go for industrial scale alpha amylase production in Bangladesh.

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Introduction

Amylases are one of the most important enzymes used in the textile industries. They also have application in a wide number of industrial processes such as food, fermentation and pharmaceutical industries.

The synthetic media used for the production of amylases have been a bit costlier and that's why researchers are now busy in searching of procedures to cut short the cost of production of amylases. Microbial enzymes are widely used now a day in industrial processes due to their low cost, large productivity, chemical stability, environmental protection, plasticity and vast availability (Burhan *et al.* 2003; Mishra and Behera 2008).

Alpha-amylase can be obtained from plants, animals and microorganisms. From barley and rice it has also been isolated (Oboh 2005), however they are not commercially viable. Microorganism can play an important role in the production of α-amylases because of their rapid growth and can be manipulated easily through biotechnology. Large number of microbial α-amylases has different applications in different industrial sectors such as food, textile, paper and detergent industries.

All α-amylases (EC 3.2.1.1) are starch-degrading enzymes that catalyze the hydrolysis of internal α-1, 4-glycosidiclinkages (Gupta *et al.* 2008; Rajagopalan and Krishnan 2008). Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market (Rajagopalan and Krishnan 2008).

Interestingly, the first industrially produced enzyme was an amylase from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders.

In addition, they are used in the removal of starch from textiles, direct fermentation of starch to ethanol production of foods with high energy (Aiyer 2005).

They are potentially useful in the pharmaceutical and fine chemicals industries if enzymes with suitable properties could be prepared (Kelly *et al.* 1980).

The spectrum of amylase application has widened in many other fields, such as clinical, medical, and analytical chemistries, as well as their wide spread application in starch saccharification and in the textile, food, fermentation, paper, brewing and distilling industries.

Fungal sources of α-Amylase are kept to global isolates, commonly *Aspergillus* species and a few species of *Penicillium*.*P. brunneum*,*P. fellutanum* were used in the previous studies to produce α-Amylase by submerged fermentation (Erdal and Taskin 2010). *P. expansum* MT-1 has been used to produce the enzyme by solid state fermentation (Balkan and Ertan 2007). *P. chrysogenum* was also used as the microbial source for producing amylase by solid state fermentation using various substrates such as, corncob leaf, rye straw, wheat straw etc. (Goto *et al.* 1998). For commercial production of α-Amylase, the fungal sources those are most commonly used are the strains of. *A. oryzae*, *A. niger* and *A. awamori* among several others (Konsoula and Kyriakides 2007). *A. fumigatus*is used for the production of the enzyme by submerged fermentation technique (Bin *et al.* 1997). Genetically modified organisms are also being used for production of α-Amylase.

Mainly two methods are used for production of α-Amylase on a commercial scale. These methods are Submerged Fermentation (SmF) method and Solid State Fermentation (SSF) method. SSF is a new method while the SmF is the traditional method of enzyme production from microbes which has been in use for an extensive period of time.

The production of alpha amylases in lower cost is essential for the boom of the economy of our country as garment industry is one of the major income generating sectors in Bangladesh. Therefore, in our project we have produced alpha amylase from the *A.*

niger in a cost effect manner. The cost of substrates on which enzyme-producing microbes can be grown has been a significant factor in the manufacture. For many such motifs, SSF is considered a hopeful method for commercial production of alpha-amylase. So, in the project, we produced alpha-amylase using wheat bran as a substrate in solid-state fermentation.

Materials and methods

Pretreatment of substrates

For low cost production of α-Amylase enzymes, we have used wheat bran as a substrate. Wheat bran was collected from the local market of Rajshahi, Bangladesh. It was first washed with cold water and then with warm water to remove dirt and impurities.

The substrate used in this study was pretreated with NaOH. In this process, the substrate was treated with NaOH (1% w / v distilled water) for 1 hour and filtered through cheesecloth and washed with water until the washing liquids became neutral. The treated samples were dried overnight in the oven at 110°C. The dried substrates were ground in a laboratory mill, autoclaved at 121°C and used for further studies (Gomes *et al.* 2006).

Collection of microorganism

Fungus was isolated form soil samples. Samples were collected from the University of Rajshahi, Bangladesh. Serial dilution was used to isolate the fungus.

The inoculated Petri plates were incubated at 28°C for 4 days (Khan and Yadav 2011). The initial fungal isolates were identified according to microscopic (Sharma and Rajak 2003) and morphological characteristics. The isolates were picked up and further inoculated on sterile potato dextrose agar plates by spot inoculation and incubated for 4 days at 28°C to obtain pure fungal plates.

Pure culture of A. niger

Spores of *A. niger* was inoculated on potato dextrose broth media at pH 6.2 it was used as seed for the culture of the fungal strain (Handajani and Setyaningsih 2006). *A. niger* was grown in a continuous shaker for 4 days with shaking at 160 rpm and 28°C.

Fermentation for amylase production

Wheat bran was used in the current experimental protocol as substrate to carry out fermentation at 28°C for 4 days to produce α-Amylase. It was used with MSM Broth as fermentation medium (Gangadharan *et al.* 2006). For SSF 20 gm of powdered wheat bran was taken in 250ml flasks and moistened with 50ml of MSM containing the following in gm/l (0.8 g NaCl , 0.8 g KCl , 0.1 g $CaCl_2$, 2.0 g KH_2PO_4 , 0.2g $MgSO_4$, 0.1 g $FeSO_4.7H_20$, 8.0 g Glucose, 2.0 g NH_4Cl, pH 6.2).

Extraction of crude enzyme

Crude enzyme was extracted from fermented media by adding 100ml distilled water with agitating the flask in a shaker at 160 rpm for 1 hour. The mixture was filtered through cheesecloth and centrifuged at 4000 rpm at 4°C for 10 min. The supernatant was collected and treated as crude enzyme (Muthezhilan *et al.* 2007; Singh *et al.* 2009).

CMC assay for determination of the cellulytic activity of A. niger

For determination of the potential cellulytic activity of the *A. niger* strain, carboxymethyl cellulase (CMC) agar plate assay was utilized. Each liter of CMC medium contained 2 g $NaNO_3$, 1 g $K_2H_2PO_4$, 0.5 g $MgSO_4$, 0.5 g KCl, 2g CMC Sodium Salt, 0.2 g peptone, 2.7 g agar, 1.0 g yeast extract. Commercially used enzyme in textile industries was used as control.

Dinitro-salicylic-acid (DNS) assay

Dinitro-salicylic-acid (DNS) assay was carried out for determination of the activity of extracted enzyme. In the dinitro-salicylic acid method, aliquots of the substrate stock solution were mixed with the enzyme solution. Followed by 10 min of incubation at 50°C, DNS reagent was added to the test tube and the mixture was incubated in a boiling water bath for 5 min. After cooling to room temperature, the absorbance of the supernatant at 540nm was

measured.

Optimization of culture conditions for enzyme production

Optimization of culture conditions is important in obtaining the highest amount of yield which depends on the microbial source, desired end product, method of fermentation employed and many other factors.

In order to get optimum production of alphaamylases b y the fungal isolate, substrate, temperature and pH optimization experiment was carried out.

For optimizing the temperature for the best growth of the isolate showing maximum hydrolysis, plates were inoculated with the fungal isolate and inocubated at variable temperatures (Laderman *et al.* 1993). Optimum pH for enzyme production was also carried out using various pH through the protocol described earlier by Bhargav *et al.* (2008).

Results and discussion

Collection and Pure culture of A. niger

A. niger was isolated form soil samples that was collected from the University of Rajshahi, Bangladesh. The culture was identified based on colony morphology and microscopic examination. Similar experiment was reported by Devanathan *et al.* (2007). *A. niger* was preserved in 4°C refrigerator, until needed.

Table 1. Cost calculation for 1 liter α-Amylase production using our local resources and minimum optimization required for setting up an enzyme industry in Bangladesh.

Parameters	Cost in BDT
Fermentation medium	38.82
Substrate cost	10.00
Water	2.00
Quality control	1.25
Maintenance	2.25
Electricity Bill	3.60
Total cost	57.92

Fermentation of A. niger on wheat bran as a cheap alternative to media

The study revealed that the cultured *A. niger* was able to ferment wheat bran (Fig. 2). Study also reveals that the high yield of alpha amylase was obtained in this fermentation. Similar result was reported by Pandey (1991). Different researcher's reveals that wheat bran is cheaply and readily available and it contains sufficient nutrients that support good microbial growth and high yield of enzymes as well (Pham *et al.* 1998; Balkan and Ertan 2007).So, wheat bran can be used as a substrate as a cheap alternative to media.

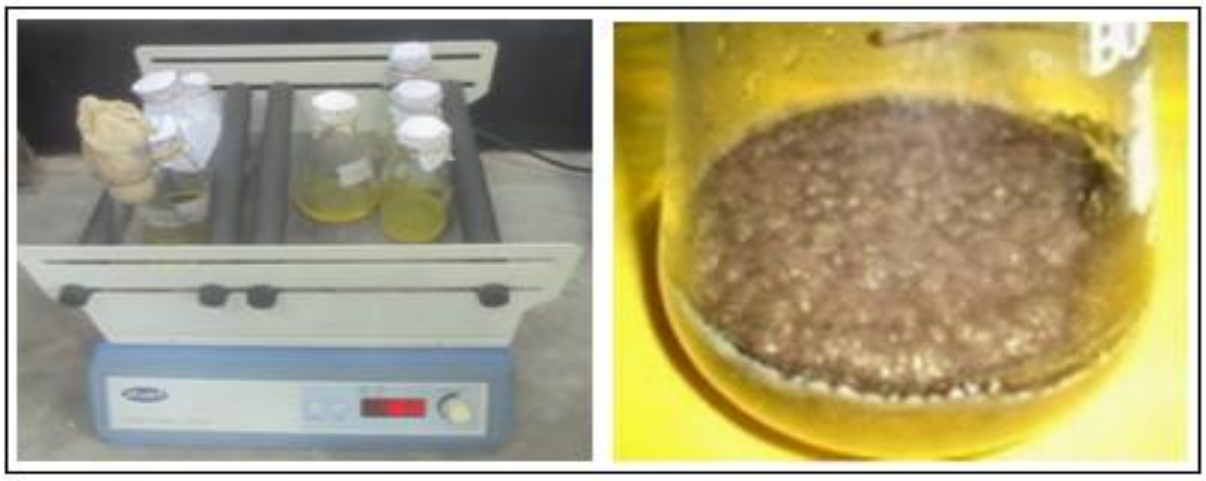

Fig. 1. Culture of *A. niger* on potato dextrose broth medium, pH 6.2 at 28°C for 4 days.

Extraction of crude enzyme

After centrifugation the supernatant was filtered through Whatmann No. 1 filter paper and filtrate was considered a crude enzyme (Lowry *et al.* 1951). Further enzyme amylase was assayed by CMC and DNS method (Ghose 1987; Bailey *et al.* 1992).

CMC assay for determination of the cellulytic activity of A. niger

CMC agar plate assay was used in this experiment to determine cellulytic activity of the microorganisms (Fig. 3).

After 48 hour incubation at 28°C temperature CMC plate was flooded with Gram's Iodine solution. A prominent hallo zone was observed in the CMC plate. So, the study reveals that *A. niger* have cellulytic activity.

Fig. 2. Fermentation of *A. niger* on wheat bran as a substrate for amylase production. Fermentation was carried out at 28°C for 4 days and pH was set at 6.2.

Dinitro-salicylic-acid (DNS) assay

Amylase activity was estimated by the analysis of reducing sugar released as a result of the action of α-amylase on starch. In this study α-amylase from the enzyme assay was carried out by measuring the reducing sugars by DNS method and activity was found to be maximum after 6 days of incubation (Fig. 3).

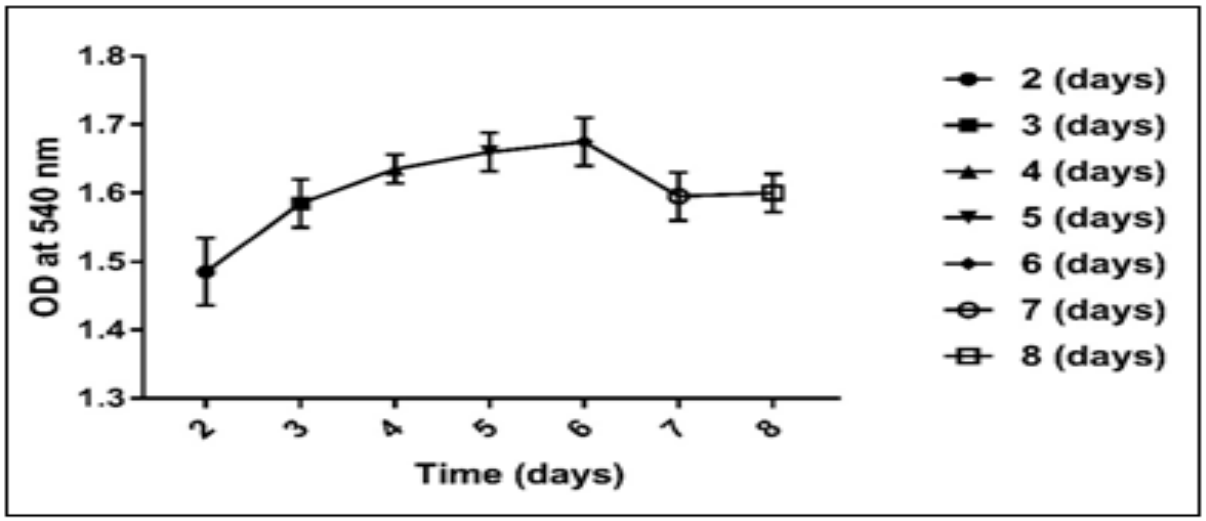

Fig. 3. DNS assay result of crude enzyme. Maximum amylase activity was found after 6 days of incubation, pH 6.2 at 28°C. OD was measured at 540nm.

Optimization of culture conditions for enzyme activity

Effect of carbon source

The composition of media plays an important role in the production of enzymes. Growth and enzyme production of any organism are greatly influenced by both environmental conditions as well as the nutrients available in the growth medium (Singh *et al.* 2011). Carbon was one of the major elements in the medium composition for the metabolic activities of the isolate. The current experimental results showed that with increasing concentration of starch substrate the enzyme activity was increased.

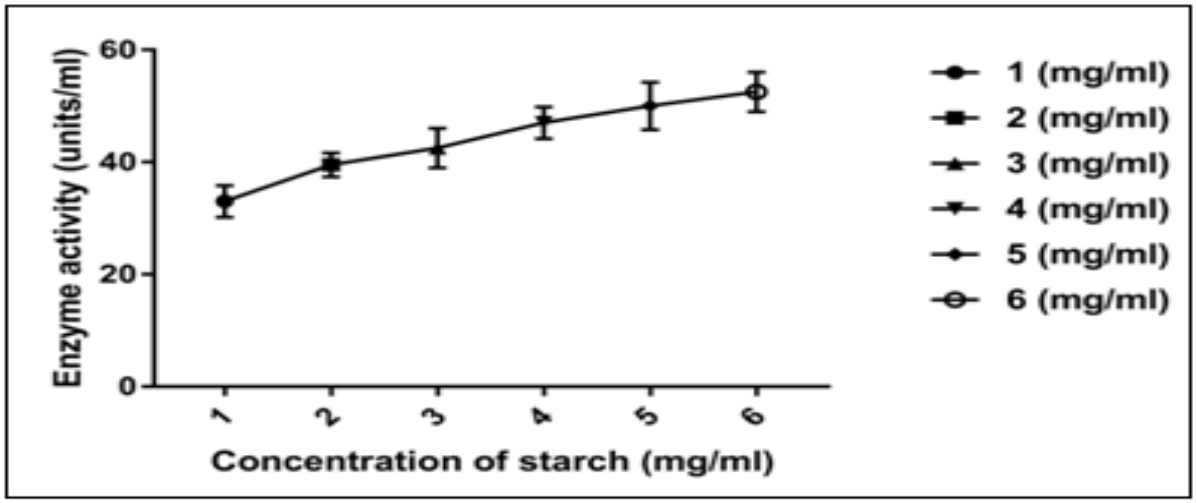

Fig. 4. Effect of starch on enzyme activity. With increasing the concentration of starch the enzyme activity is increased. Experiment was carried out at 28°C and pH 6.2.

The maximum production of enzyme was found at 6 mg/ml starch concentration (Fig. 4).

Similar results of catabolite repression of enzyme production by glucose, has been reported by Nandakumar *et al.* (1999) for *A. niger* CFTRI 1105 and Bhavya (2007) for *Aspergillus* sp. JGI 12.

Optimization of temperature and pH for maximum enzyme production

The enzyme production was increased with increase in temperature till it reaches the optimum. The results are shown as the activity of the crude enzyme extracted from cultures fermented at different temperatures and pH.

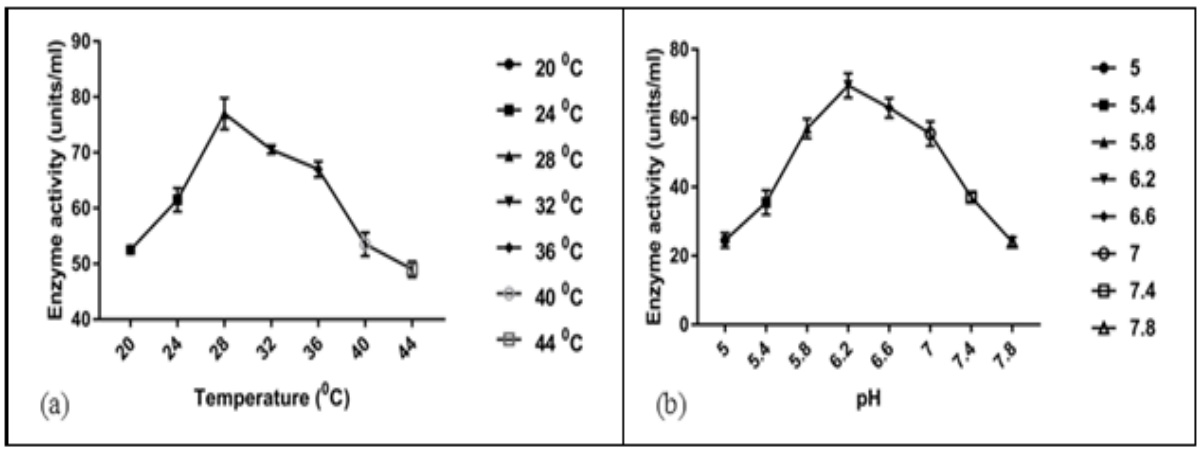

Fig. 5. Optimization of temperature and pH for maximum enzyme production. (a) Effect of temperature on enzyme activity. Maximum enzyme activity was found at 28°C. (b) Effect of pH on enzyme activity. Maximum enzyme activity was found at pH 6.2.

After reaching its optimum, with increasing the temperature then the enzyme activity was decreased. The maximum enzyme activity was found at 28°C (Fig. 5a). Kathiresan and Manivannan (2006), reported 30°C to be the best for enzyme production by *Penicillium fellutanum*. In our current project maximum yield of enzyme (as shown by enzyme activity) was gained at pH 6.2 (Fig. 5b). Similar findings were reported by Shinde *et al.* (2014) who found maximum enzyme activity by *A. Niger* and *Bacillus licheniformis* at pH 6.0 and Ellaiah *et al.* (2002) reported that *A. niger* UO-01 had a preference to pH around 6.0 for amylase production but its production capacity decreased for pH levels higher and lower, probably as a consequence of a reduction in the metabolic activity of the amylase producing strain.

Cost calculation

Prime objective of this project was to establish a protocol for the low cost production of α-Amylase enzymes for textile industries in Bangladesh. Since currently in Bangladesh textile industries totally depend on imported enzymes, we target to produce α-Amylase using our local resources. Commercial α-Amylase enzymes which are used now-a-days costs about BDT 250-350 per liter.

Our cost calculation suggests that we will be able to produce α-Amylase using our local resources at BDT 57.92 per litre which is shown in Table 1.

Conclusion

Based on the above study, it can be concluded that wheat bran can be a very good substrate for the production of alpha-amylase and can help to reduce production cost.

We are optimistic to set up a protocol for the production of α- Amylase enzyme in industrial scale with promising cost-effective procedure.

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