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USAGE OF SUGAR CANE BAGASSE AS AN ENERGY SOURCE FOR THE PRODUCTION OF LIPASE BY *ASPERGILLUS FUMIGATUS*

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Abstract

The sugar cane industry residue/waste such as sugar cane bagasse contains complex lignocellulosic material, which can be used as an ideal and low cost carbon and energy source for the growth of microbes and production of microbial metabolites under submerged fermentation after pretreatment process**.** The sugar cane bagasse was pretreated with acid/base (0.3N and 0.6N H_2SO_4, NH_4OH; 2.5% and 5.0% H_2O_2) to fermentable sugars. These fermentable sugars were used with mineral medium for the growth of *Aspergillus fumigatus* and production of industrially important enzyme lipase. *Aspergillus fumigatus* secreted maximum production of lipase 40.0 U/ml at 48 hours cultivation time, when it was grown on city sugar cane bagasse hydrolysate (pretreated with 0.6N NH_4OH) mineral medium in comparison to industrial bagasse and other acid/base hydrolyzed sugar cane bagasse. The final pH of culture broth, reducing sugar and lipase activity were checked from fermented medium

Introduction

Recently, the use of alternative substrates for the production of high value products presents an advantage over the traditional processes. The recycling of material in which material are systematically used and reused to bring about the drastic increase in resource productivity needed to make human activity sustainable (Clift, 1997; Cammarota & Freire, 2006). Several species of bacteria, yeast and fungi are well known to secrete lipases during growth on hydrophobic recycled low cost substrates, which are important sources for industrial applications. Lipases are usually extracellularly produced either by submerged or by solid-state fermentation (De-Azeredo *et al*., 2007; Iftikhar *et al*., 2010).

Lipases (E.C. 3.1.1.3) are carboxyl esterases that catalyzed the hydrolysis of fats and oils at the oil-water interface to glycerol and free fatty acids. The industrially important microbial lipases were found to be particularly useful for spectrum of transesterification and other reactions (Harwood, 1989; Malcata *et al.*, 1992; Dandik *et al*., 1993; Jaeger *et al*., 1997; Jaeger & Reetz, 1998; Shimizu & Nakano, 2003) and other numerous applications such as ester synthesis, production of biosurfactants (Chopineau *et al*., 1988) and preparation of enantiomerically pharmaceuticals (Hiol *et al*., 2000). Microbial lipases have attracted considerable attention owing to their biotechnological potential, ranging from the use in laundry detergent to stereospecific biocatalysis (Jaeger *et al*., 1996; Asad *et al.*, 2011). Nowadays, the uses of lipases have been increased in the food, detergent, agrochemical, oleochemical, leather and cosmetics industries. Recently the role of lipase in paper and pulp manufacture has been emphasized (Fujita *et al*., 1992; Hata *et al*., 1996; Liese *et al*., 2000).

The aim of present investigation was to produce lipase by *Aspergillus fumigatus*, using pretreated sugar cane bagasse as a carbon and energy source under submerged fermentation.

Materials and Methods

Hydrolysis of sugar cane bagasse and isolation of micro organisms: Sugarcane bagasse was collected from city sugarcane peeler and sugar industry Khoski, which was hydrolyzed with acid and base as described previously (Naqvi & Dahot, 2000). The sugarcane bagasse hydrolysate was used as a carbon and energy source in the mineral medium.

Aspergillus fumigatus was isolated from soil of University of Sindh, Jamshoro and identified in Enzyme and Fermentation Biotechnology Research Laboratory, Institute of Biotechnology and Genetic Engineering. The identified *Aspergillus fumigatus (EFB-7)* was preserved. The Stock Culture was maintained on Czepaks agar as described previously (Naqvi & Dahot, 1998).

Preparation of spore suspension: The surface of stock culture of *Aspergillus fumigatus* was gently rubbed with sterilized wire loop and 10 ml of sterilized distilled water was added. The spore suspension was further diluted to 100 ml with sterilized water as reported by Schwermann *et al*., (1994).

Culture condition: The following ingredients were used for the preparation of fermentation medium as reported by Burrel *et al*., (1966). The fermentation medium composed of (g/L) of KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.2; $CuSO_4.5H_2O$, 0.0023; $FeSO_4.7H_2O$, 0.0063; $ZnSO_4.7H_2O$, 0.0011; $MnCl_2.4H_2O$, 0.0035; $CaCl_2.2H_2O$, 0.0467 and NH_4NO_3, 2.4 was used for the production of Lipase. The initial pH of the medium was maintained at 6.0 (Enari, 1983).

Fifty ml of fermentation medium containing sugarcane hydrolysate was taken in 250 ml conical flask and the initial pH of the medium was maintained at 6.0. Flasks were plugged with cotton wool and were autoclaved at 1.5kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature, was inoculated with 1.0ml spore suspension of *Aspergillus fumigatus*. These flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 30±2ºC. The culture broth was separated from mycelium after an interval of 24 hours incubation period through Whatman No: 1 filter paper.

Biomass estimation: The culture broth was filtered through pre-weighed Whatman No. 1 filter paper. The filter paper containing biomass was washed with distilled water and dried at 105-110°C in an oven and its dry weight was noted. The culture filtrate was used as the source of lipase.

Lipase assay: Lipase activity was measured by the olive oil emulsion method (Official Methods of analysis, 1965; Dahot & Memon, 1987). The reaction mixture containing 2.5ml of substrate (10% olive oil emulsion with 2% gum Arabic in 0.2M sodium phosphate buffer) and 2.5 ml enzyme solution was incubated at 37°C for 1 hour in shaking water bath incubator at 200 rev. /min. The reaction was stopped by the addition of 5.0ml methanol: chloroform (2: 1 v/v).

A unit of lipase activity was defined as the amount of lipase required to release one micro mole of free fatty acids per hour under the assay conditions.

Determination of reducing sugars: The reducing sugar concentration in the culture broth was determined by Dinitrosalicylic acid (DNS) method at 450nm (Miller, 1959) and the results were calculated from glucose standard curve.

Results and Discussion

In this study, we have tried to utilize sugarcane bagasse waste, which was collected from city sugarcane peeler (named as city bagasse) and also collected from sugarcane industry (named as industry bagasse) and to develop a low cost process for the production of industrially important lipase enzyme by *Aspergillus fumigatus*. In this study, sugarcane bagasse (city sugar cane bagasse and industry sugar cane bagasse) were hydrolyzed with 0.3N, 0.6N H_2SO_4, NH_4OH and 2.5%, 5.0% H_2O_2 to fermentable sugars and these fermentable sugars were supplemented with mineral medium for the growth of *Aspergillus fumigatus* and production of lipase. It is reported that release of fermentable sugars varies from acid to acid and carbon source to carbon source used in hydrolysis process (Han & Callihan, 1974; Han & Auderson, 1975; Rivier, 1977). The chemical pre-treatment method is less expensive and more effective (Han *et al*., 1978; Vaccarino *et al*., 1989) than physical treatment method.

The lipase production pattern by *Aspergillus fumigatus* was grown on 0.3 N H_2SO_4 treated city and industry bagasse hydrolysates presented in Figs 1-2. It is clear from the result that the higher rate (26.75 Units/ml) of lipase production was obtained at 48 hours in sugar cane bagasse collected from city. The amount of reducing sugars contents in the culture medium decreases with the passage of time in both substrates. In case of final pH and biomass the rate was slightly increased with the increase of incubation time in both cases.

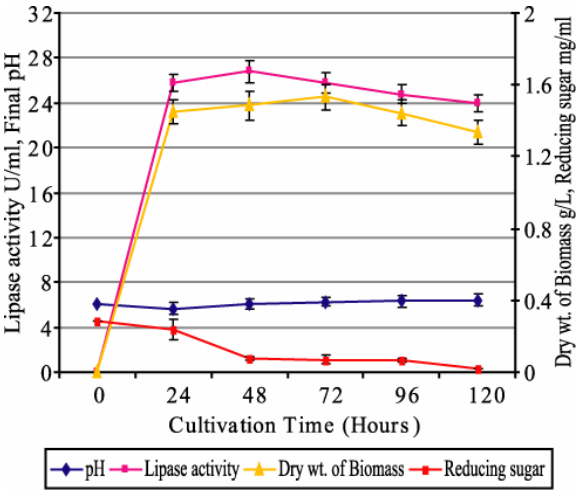

Fig. 1. Lipase production by *Aspergillus fumigatus* grown on 0.3N H_2SO_4 pretreated sugar cane bagasse collected from city sugar cane peeler.

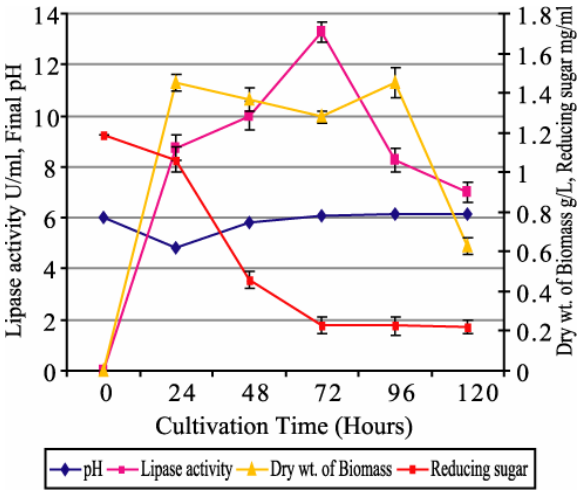

Fig. 2. Lipase production by *Aspergillus fumigatus* grown on 0.3 N H_2SO_4 pretreated sugar cane bagasse collected from sugar cane industry.

The higher yields of lipase were found at 72 hours at 30±2°C by *Aspergillus fumigatus* grown in city and industry sugar cane bagasse hydrolysate (treated with 0.6 N H_2SO_4) mineral medium (Figs. 3-4). The amounts of reducing sugars in the culture broth continuously decreased with the increase of time period (city & industry bagasse). The pattern of final pH of broth and mycelial biomass were similar as mentioned in previous data.

The lipase activity reached highest at 72 hours at 30±2°C when *Aspergillus fumigatus* was grown on 0.3 N NH_4OH treated city and industry sugar cane bagasses. During the fermentation period, the concentration of reducing sugars was decreased while final pH of broth and weight of mycelial biomass were increased as shown in Figs. 5-6.

According to the data of Figs 7-8, the rate of lipase activity increased up to 48 hours in city bagasse (approximately 40.0 U/ml) but at 72 hours in case of industrial sugar cane bagasse then declined, when both bagasses were hydrolyzed with 0.6 N NH_4OH. The mycelial biomass is activated with the increasing of fermentation time in city bagasse but slight variation was noted in case of industry bagasse. On the other hand, the amount of reducing sugar contents in culture medium decreased during the batch wise submerged fermentation process and the final pH was increasing with every interval of 24 hours.

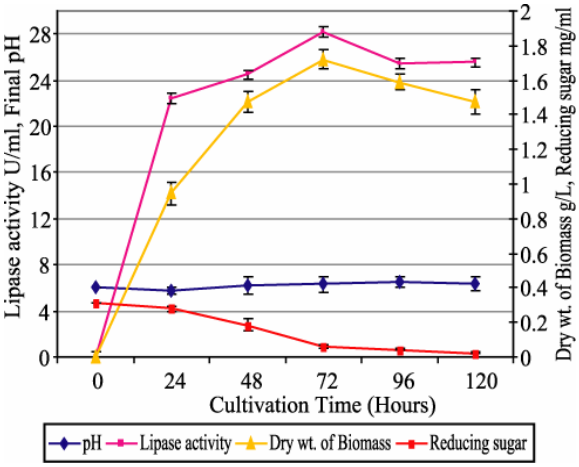

Fig. 3. Lipase production by *Aspergillus fumigatus* grown on 0.6 N H_2SO_4 pretreated sugar cane bagasse collected from city sugar cane peeler.

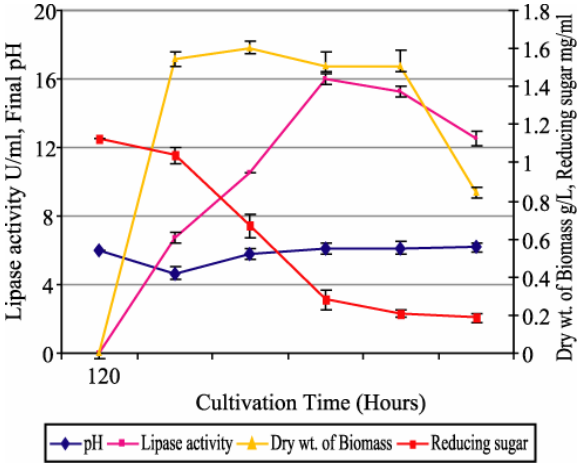

Fig. 4. Lipase production by *Aspergillus fumigatus* grown on 0.6 N H_2SO_4 pretreated sugar cane bagasse collected from sugar cane industry.

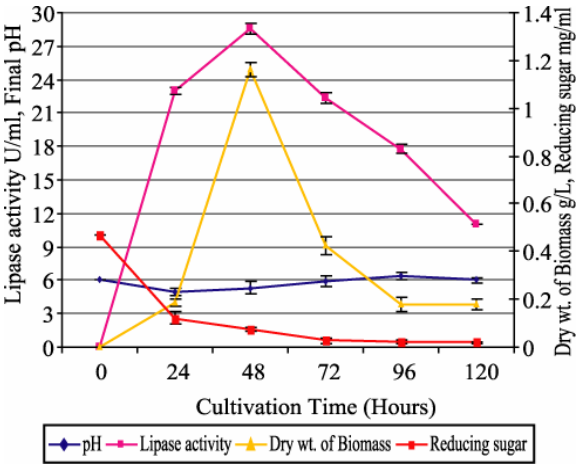

Fig. 5. Lipase production by *Aspergillus fumigatus* grown on 0.3 N NH_4OH pretreated sugar cane bagasse collected from city sugar cane peeler.

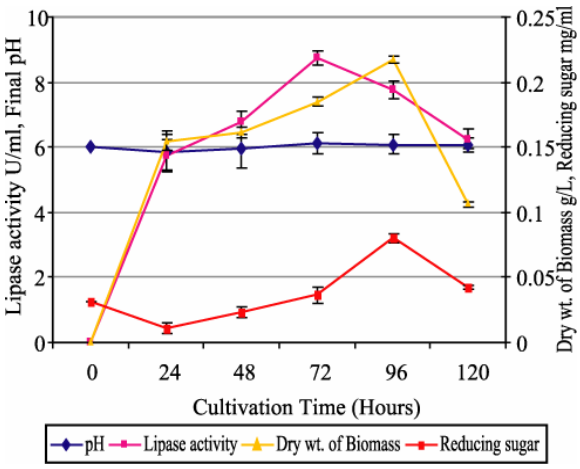

Fig. 6. Lipase production by *Aspergillus fumigatus* grown on 0.3 N NH_4OH pretreated sugar cane bagasse collected from sugar cane industry.

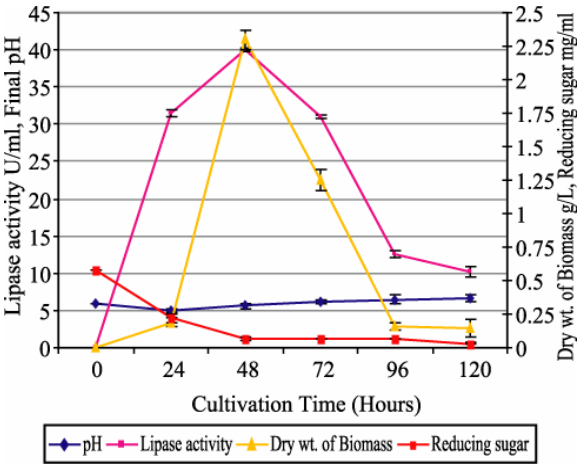

Fig. 7. Lipase production by *Aspergillus fumigatus* grown on 0.6 N NH_4OH pretreated sugar cane bagasse collected from city sugar cane peeler.

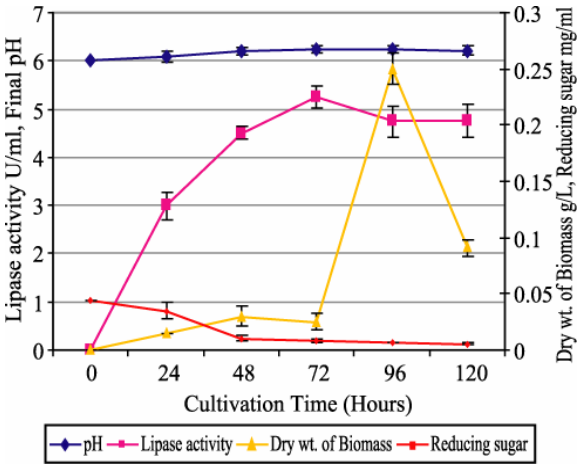

Fig. 8. Lipase production by *Aspergillus fumigatus* grown on 0.6 N NH_4OH pretreated sugar cane bagasse collected from sugar cane industry.

City and industry bagasses were treated with 2.5% H_2O_2 and the hydrolysate was supplemented with mineral medium and inoculated with *Aspergillus fumigatus* for the synthesis of lipase and the results are depicted in Figs 9-10. The maximum production of lipase and growth of microorganisms were achieved at 48 hours in city bagasse but in industry bagasse, the higher yield of lipase and protein were found at 24 hours, and after this period lipase activity declined. The concentration of reducing sugar decreases after the passage of incubation time. In early stage of growth the pH of fermented medium was increased up to 7.0 than slightly decreased towards the acidic side.

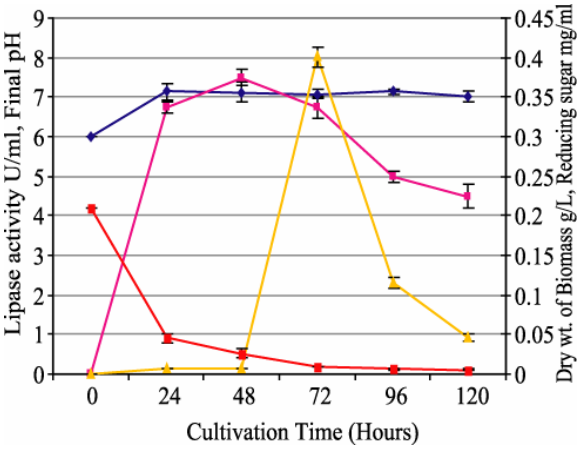

Fig. 9. Lipase production by *Aspergillus fumigatus* grown on 2.5% H_2O_2 pretreated sugar cane bagasse collected from city sugar cane peeler.

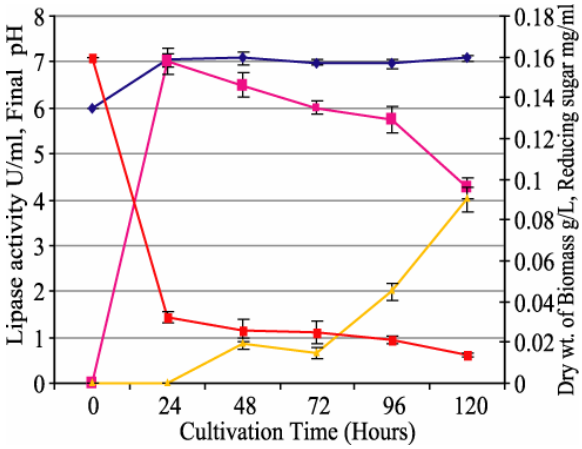

Fig. 10. Lipase production by *Aspergillus fumigatus* grown on 2.5% H_2O_2 pretreated sugar cane bagasse collected from sugar cane industry.

Aspergillus fumigatus was inoculated in hydrolysate of city and industry sugar cane bagasses, which were treated with 5% H_2O_2 as shown in Figs 11-12. The log phase of growth reached up to 48 hours in city bagasse and up to 24 hour in industry bagasse and enters into stationary phase for the higher production of lipase, whereas the pH value of broth turned to near to neutral side through out the fermentation period in both cases. The fluctuation can be noted in growth of mycelial mass in city bagasse but increases in industry bagasse. On the other hand, the concentration of reducing sugar decreased with the increase of time.

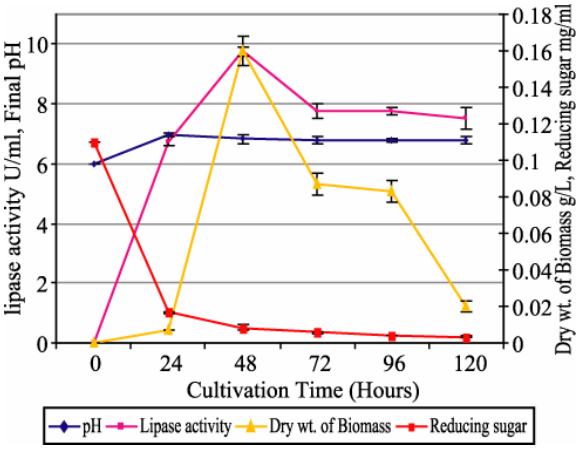

Fig. 11. Lipase production by *Aspergillus fumigatus* grown on 5% H_2O_2 pretreated sugar cane bagasse collected from city sugar cane peeler.

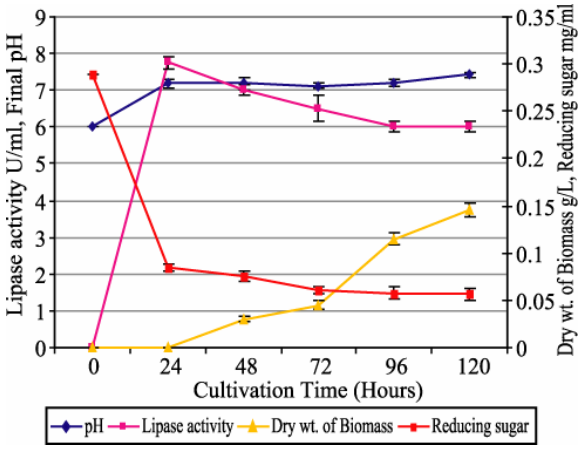

Fig. 12. Lipase production by *Aspergillus fumigatus* grown on 5% H_2O_2 pretreated sugar cane bagasse collected from sugar cane industry.

Fig. 13 shows the comparison of over all lipase production by *Aspergillus fumigatus* grown on different pretreated sugar cane bagasses. It is clearly seen that higher yield of lipase was achieved in hydrolysate of city sugar cane bagasse, which was incorporated with mineral medium treated with 0.6N NH_4OH.

Haba *et al*., (2000) have reported that maximum production of lipase was recorded 2748 U/l and 1703 U/l by *Pseudomonas* sp., *3AT* and *Pseudomonas aeraginosa ATCC-111*, when grown on waste frying oil mineral medium. The present results are also in harmony with the observation of other researchers in the field of lipase production (Gulati *et al*., 1999; Maia *et al*., 1999; Ternerler & Keshavarz, 2000; Iftikhar *et al.*, 2011).

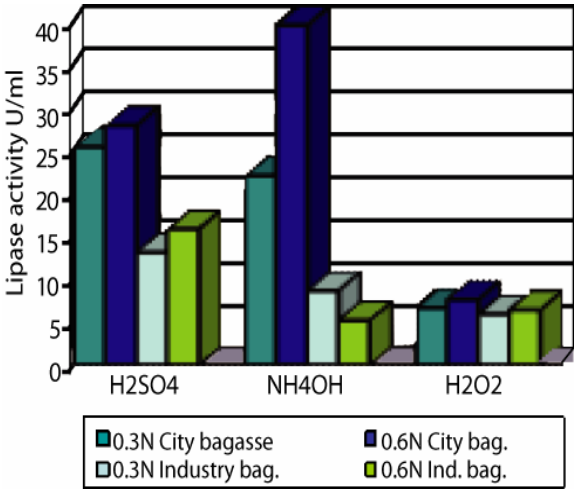

Fig. 13. Comparison of lipase production rate by *Aspergillus fumigatus* grown on pretreated sugar cane waste.

Conclusion

It is concluded that higher yield of lipase (approximately 40.0 Units/ml) was observed by *Aspergillus fumigatus*, when it was grown on 0.6N NH_4OH pretreated city sugar cane bagasse mineral medium at 48 hours in comparison to other pretreated acid/base sugar cane waste. On the basis of higher production of lipase, it is suggested that the hydrolysate of city sugar cane bagasse is the best and cheapest source for the production of lipase.

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