Effect of temperature on cellulose enzyme activity in crude extracts isolated from solid wastes microbes

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Abstract- Cellulose is degraded by three major classes of hydrolases. These three classes have been isolated and purified from fungi. They range widely in temperature and pH optima, but the most common enzymes are most active at pH 5.5, 55°C.Three cellulases are of interest: 1) endoglucanase (carboxymethyl cellulase or CMCase), 2) cellobiohydrolase (CBH or filter paper activity) and 3) β-glucosidase. Endoglucanase (CMCase) attacks randomly in the interior of the cellulose structure. It is not very active against crystalline cellulose, but they are capable of hydrolyzing substituted cellulose such as carboxymethyl cellulose. It produces cellulodextrins also known as cellulooligosaccharides. Cellobiohydrolase (CBH) also known as exocellulase attacks crystalline cellulose from the non-reducing end and produces cellobiose. β-glucosidase hydrolyses cellobiose to glucose.
Key words: Cellulose, Cellobiohydrolase, hydrolases.

Introduction

"Solid waste" means any garbage, refuse, sludge from a waste treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, including solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining, and agricultural operations, and from community activities. Organic waste is a major component of municipal solid waste. Municipal Solid Waste (MSW) compost contains a significant amount of humic substances. (wei.z *et al*., 2007).In India, the amount of waste per capita generated is estimated to increase at a rate of 1 – 1.33 % annually (Shekdar 1999), scenario assuming the daily per capita waste generation in 1995 as 0.456 kg , and the per capita increase in waste generation as 1.33%. The calculated value of daily per capita waste generation in 1997 is 0.468 kg. It is evident that the total waste quantity generated in 2047 would be approximately above 260 million tones, more than five times the present level. Further a wide variety of pathogenic microorganisms have been reported to be present in these organic wastes. (Amalraj,S et al., 2006).Attempts were made to extract cellulase enzyme from solid waste microflora

Materials and Methods

Inoculation of sample by spread plate method

The fungal samples were collected from various sources of solidwaste viz. straw, orange peel and wood source: Black color spores were seen on strawb)A grey-green growth was seen on orange peel. white colored fungal growth was seen on wood surface. The fungal samples were added to 10 ml sterile distilled water in 3 different test tubes labelled as black, grey and white for a, b, and c respectively. From each of the test tubes 0.5 ml volume was pipetted onto the PDA media on 3 separate petri plates. The petri plates were labelled accordingly. Using L-shaped glass rod the sample was uniformly distributed. The petri plates were incubated for 3 to 4 days in an inverted position at room temperature.

Isolation and subculture

After incubation appearance of the discrete, well separated colonies was examined, the next step was to subculture some of the cells from the colonies to separate agar plates with a sterile needle or loop for further tests and experiments [4]. Each of these new cultures represents the growth of a single species and is called pure or stock culture.

Identification of fungi using cotton blue stains

Place a drop of distilled water on a clean slide. Transfer a small tuft of the fungus, preferably with spores and spore bearing structures into the drop using a flamed, cooled needle. Gently tease the material using the two mounting needles. Add a drop of lacto phenol cotton blue stain over the material. Gently mix the stain with the mold structures. Place a cover slip over the preparation taking care to avoid air bubbles in the slide. Examine slides under low and high power objectives of the microscope. The fungal species were identified based on their mold structures as viewed under the microscope.

Extraction of crude enzyme from fungi

We had previously weighed the empty Whatman filter papers. Subject the filter paper with its contents (fungal mycelium) for drying in a hot air oven at 105 ° C for 48 hours. Weigh the dried filter papers with its contents and note down the weight. Keep the filter paper with mycelium in the oven once again for 2 more hours and weigh it once again. If there is no

difference in weight, it indicates that the mycelium is completely dry. Subtract the empty filter paper weight from the weight of the filter paper with mycelia. Express the weight of the mycelium in gm/litre of culture.

Quantitative estimation of protein by Bradford's method in the culture [2]. This is a rapid, simple and sensitive method for estimation of proteins in sample extract. The color development is virtually complete within 2 minutes and the color is stable for about 1 hour. Unlike Lowry's method, ions such as NH_4^+, Na^+, K^+, phenols and carbohydrates such as sucrose do not interfere in this assay. This procedure is based on interaction of a dye, Coomassie Brilliant Blue, with proteins. The unbound dye has absorbance maxima at 495 nm. However, on interaction with proteins, the dye turns blue and its absorbance maxima are displaced to 595 nm. Thus from the absorbance at 595 nm the amount of protein in a sample solution can be quantitatively estimated. However, as in Lowry's procedure, detergents such as SDS, Triton X-100 etc., interfere in estimation of proteins by this method.

Filter paper activity(FPA) for total cellulose activity in crude extracts

Take 3 ml of crude cellulase in 3 test tubes Whatman No.1 filter paper strip (1×6 cm; 50 mg) was immersed in the enzyme in each of the test tubes.1 ml of buffer was added to each of the test tubes. Blank was prepared by taking 3 ml of distilled water instead of crude enzyme in a test tube and adding filter paper and 1 ml of buffer to it. Incubate the test tubes at 50 ° C for 1 hour in water bath. Remove the test tubes and add 2 ml of DNSA to each of the test tubes. Place the test tubes in water bath at 70 ° C for 10 minutes. Cool the test tubes. Set the colorimeter to zero using blank. Measure the OD at 540nm.one unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1µmole of reducing sugar from filter paper per ml per minute.

Results and discussion

We observed a rather broad temperature optimum in the range 40℃-80℃ for the cellulase produced by the three fungal species. However, the enzyme was found to be active even at 100℃ and some enzyme activity was also retained at 10℃.Optimum temperature for maximum cellulase activity was found to be 40℃ for *Fusarium* and *Penicillium* whereas it was 60℃ for *Aspergillus*. In a study carried out by Immanuel et al. [5], the enzyme production by *A. niger* was less at 20℃ (0.162 ± 0.002 IU ml^{-1}), further it increased to 0.170 ± 0.005 and 0.204 ± 0.002 IU ml^{-1} at 30 and 40℃ respectively. Finally it reached to a maximum at 50℃ (0.274 ± 0.003 IU ml^{-1}). The maximum amount of enzyme production (0.272 ± 0.003 IU ml^{-1}) was recorded at 50℃ by *A. fumigatus*. From the temperature 20 to 40℃, it was 0.156 ± 0.003, 0.184 ± 0.002 and 0.206 ± 0.003 IU ml^{-1} respectively.

Conclusion

Cellulose is the most abundant organic source of food, fuel and chemicals. However, its usefulness is dependent on its depolymerisation to fermentable sugar using microbial system. Many fungi secrete cellulase that exhibit the ability to degrade cellulose. When the fungi *Aspergillus, Fusarium* and *Penicillium* were grown on a medium containing cellulose (E.g., CMC, filter paper, etc.) , they were capable of producing cellulase enzyme. The crude cellulase extract was isolated from these fungi and a study on the biochemical characteristics of the enzyme was conducted. Cellulase has large scale industrial and environmental applications and thus the fungal sources can be tapped for large scale production of the enzyme. The production of fungal cellulase was found to be dependent on a number of factors *viz.*, substrate, substrate concentration, temperature, pH, incubation period, inorganic ions etc. and varies from specie to specie. Large scale production of cellulase for commercial purposes requires for identification of high yielding fungal sources and optimization of process conditions. In recent years, high cellulase yielding mutant and recombinant varieties have also been developed. Present study was aimed at identifying the highest yielding genus among the three test organisms used and also determining the optimum conditions required for maximum cellulase activity.

References

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1. *Aspergillus*

Weight of empty Whatman filter paper: 1.056 g
Weight of filter paper with mycelium : 1.245 g
Therefore, weight of mycelium = 1.245-1.056
= 0.189g/250 ml of culture
= 0.756g/lit of culture

glucose and protein estimation IN crude EXTRACTS

1. Calculations

Concentration of unknown = (OD of the unknown/OD of the standard) × concentration of standard

For,

Aspergillus sp.

Concentration of unknown sugar = (0.28/0.23)×0.5
= 0.6086 mg/ ml glucose
= 608.6 µg/ml glucose
= 121.72 µg/ml/min

Activity = (121.72 µg/ml/min)/ (mol. wt of glucose)
=0.676µmol/ml/min

Concentration of unknown protein = (0.28/0.23)×0.1
= 0.1217 mg/ml

Specific activity of *Aspergillus* sp = (0.676l/ml/min) / (0.1217 mg/ml)
= 5.55 µmol/mg of protein

Similarly,

Scale

X- axis: 1 cm= 0.2 mg/ml

Y- axis: 1 cm =0.05 OD

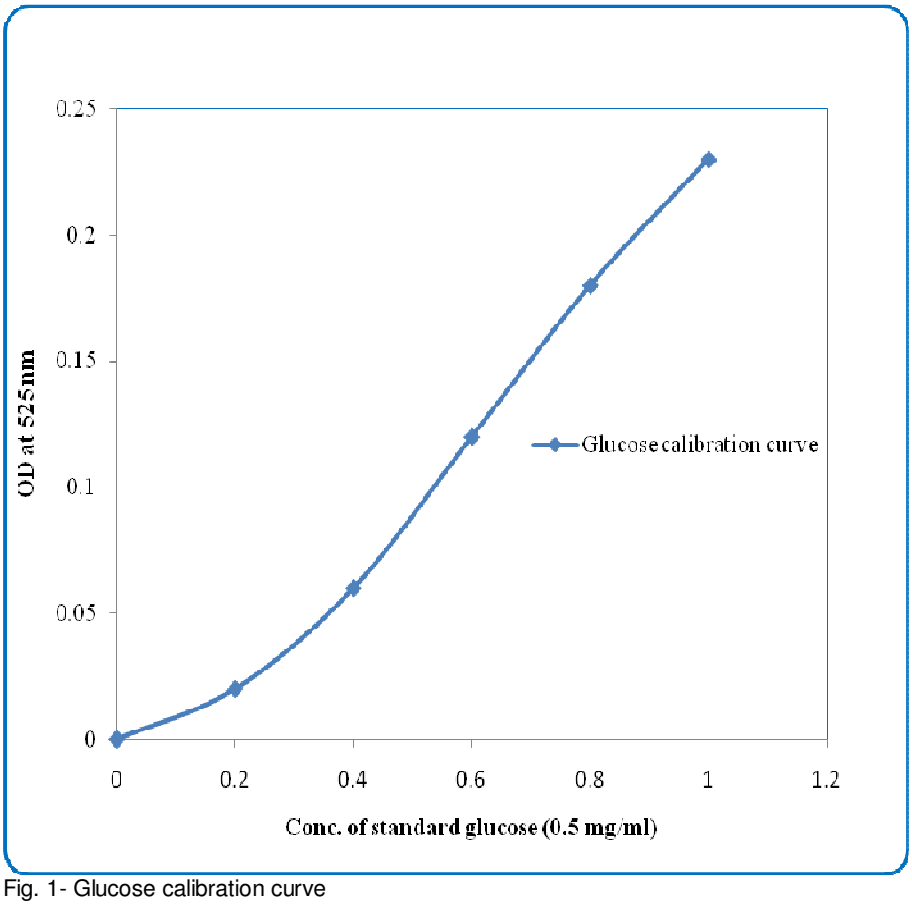

Fig. 1- Glucose calibration curve

The optimum temperature for the activity of the enzymes of the three fungal strains was found to be *Aspergillus* sp. 60℃, *Penicillium* sp. 40℃, *Fusarium* sp. 40℃

Scale:

X –axis: 1 cm = 20℃

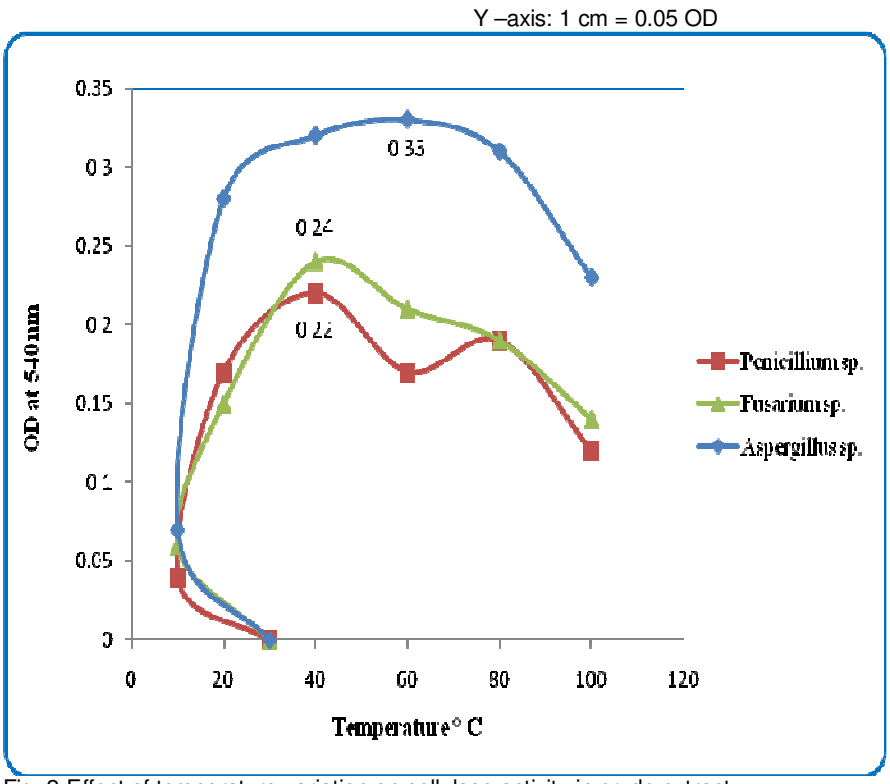

Fig. 2-Effect of temperature variation on cellulase activity in crude extract