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Short Communication

Optimization and Production of Cellulase from Agricultural Waste

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Abstract

This work represents the optimization of agricultural waste the production of cellulase using filamentous fungi Myrothecium verrucaria has been adopted. Solid sate fermentation a potential technology for production of cellulase utilizing agricultural residues. All experiments were carried out in triplicate and mean values are reported. The appropriate incubation period is one of the important parameters for cellulase production in solid state fermentation and is governed by microorganism. The result shows that optimum incubation period is important factor for production of cellulase from agricultural waste. The maximum cellulase activity has been recorded after 3 days of fermentation.

Keywords: Cellulase, fungus, fermentation, incubation period.

Introduction

Cellulase is a hydrolase enzyme which is highly applicable enzyme in textile industry, laundry detergents, pulp and paper industry for various purposes, and even used for pharmaceutical applications. The production of cellulase has been widely studied in submerged fermentation, but the high cost of enzyme production is typical in the industrial application of cellulose bio-conversion. Cellulase is an important enzyme for conversion of lignocellulosic biomass to bio-ethanol[1]. Solid state fermentation is a potential technology for production of cellulases utilizing agro-industrial residues as solid substrate due to low capital investment, low energy requirement, eco-friendly operation, and higher yield compared to submerged fermentation and lower chance of contamination due to low moisture level[2] Cellulolytic enzymes were separated by microbial degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi[3]. Pothyraj et al[4] have enhanced production of cellulase from cassava waste from various fungal cultures. Large numbers of microorganism are capable for producing of celulase but few of them produces significant quantities of enzymes[5]. Many microorganisms have the capacity to degrade these cellulosic wastes. *Myrothecium verrucaria* is a plant pathogen and it is common throughout the world, often found on materials such as paper, textiles, canvas and cotton[6]. Literature reveals that there is less work done on cellulase production from solid state fermentation of agricultural waste using *M. verrucaria*. Present research is based on production of cellulase using *M. verrucaria* from solid state fermentation of various agro waste. In present investigation filamentous fungi like *Myrothecium verrucaria* have been used for production of cellulase from solid agricultural waste. Present manuscript records optimization and production of cellulase from agricultural waste. Present manuscript records production of cellulase from agricultural waste and optimization of process parameters like temperature, incubation period, hydration, pH and weight of substrate.

Material and Methods

Spores of *M. verrucaria* have been cultivated on 2% potato dextrose agar (PDA) and incubated at 30°C for 7 days. The isolated form of white colonies containing sporodochia with a flattened or convex spore mass and one celled conidia on cylindrical, bundled phialides.

Solid substrate used: Agricultural solid waste is most cellulose abundant in nature. The agro solid waste like sugarcane bagasse, cotton seeds, cabbage, cauliflower, wheat bran and dry coconut leaves are obtained from local market of Gwalior city in month of April for the present research work of cellulose production by solid state fermentation process using micro organism *M. verrucaria*. Fermentation process and fungal spore suspensions were made using sterile water and were added to the sterile solid substrates. Flasks were incubated for 5 days in the B.O.D incubator at 30^0C.

Extraction process: Cellulose extracted by adding 50mL of double distilled water to fermentation broth and kept it for overnight at 4^0C in incubator. After incubation contents were filtered through cheese clothes and centrifuged at 10,000 rpm for 30minutes. The cellulose contents supernatant was kept at 4^0C before used.

Analysis method: Filter paper activity for total cellulase present in the culture supernatant was determined according to the method recommended by Ghose[7]. Appropriately diluted culture supernatant was added to 4 ml sodium acetate buffer (pH 4) containing 100 mg of Whatman No.1 filter paper

(Sigma–Aldrich, St. Louis, MO) strips. After incubation for 1 h the reducing sugar released was estimated by the dinitrosalicylic method[8]. 1 unit (U) of filter paper activity was defined as the amount of enzyme releasing 1 µmol of reducing sugar from the filter paper/min/g dry solid.

Results and Discussion

Cellulase production: *M. verrucaria* was initially grown on the surface of PDA slants at 30°C for 7 days. The fermentation process was carried out in 250- ml conical flasks. Separate flask was used for all six substrates. Each flask was filled with 5 g of solid substrate followed by the addition of 3 ml of water. Then the flasks were plugged with cotton and autoclaved at 121°C for 15 min at 15 psi. Under aseptic conditions fungal spores were transferred from culture slants to the solid substrate and mixed thoroughly. Then the flasks were incubated in incubator maintained at 30°C. The extra- cellular enzyme was extracted by soaking the fermented solid material with 50 ml of sterile water overnight at 4°C and filtering through muslin cloth. All six filtrates were centrifuged at 10,000 rpm for 30 min and temperature has been maintained 4°C. The supernatant was used to measure the amount of cellulase produced. Optimization of incubation period influencing cellulase yield. An experiment with different incubation periods was executed in 250ml conical flasks at 30°C. All experiments were carried out in triplicate and the mean values are reported.

Assay of cellulase activity: Filter paper activity (FPA) for total cellulase activity in the cultural filtrate was determined according to the method of Mandels et al[9]. Appropriately diluted culture filtrate as enzyme source was added to Whatman No.1 filter paper strip (1 X 6 cm; 50mg) immersed in one milliliter of 0.05 M sodium citrate buffer of pH 4.0. After incubation at 50°C for 1 hour, the reducing sugar released was estimated by dinitrosalicylic acid (DNS) method[8].

Statistical analysis: Optimization of incubation period on sugarcane bagasse and wheat bran as a substrate showing maximum activity after 3 days. Activity decreases if kept for long time. Figure-1 and figure-2 showing activity of different day period and agricultural substrates. Maximum crude cellulase activity was recorded after 3 days of fermentation.

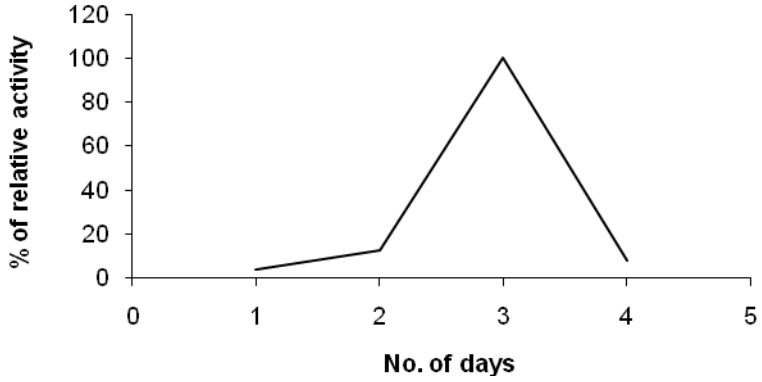

Figure-1
Activity on different days

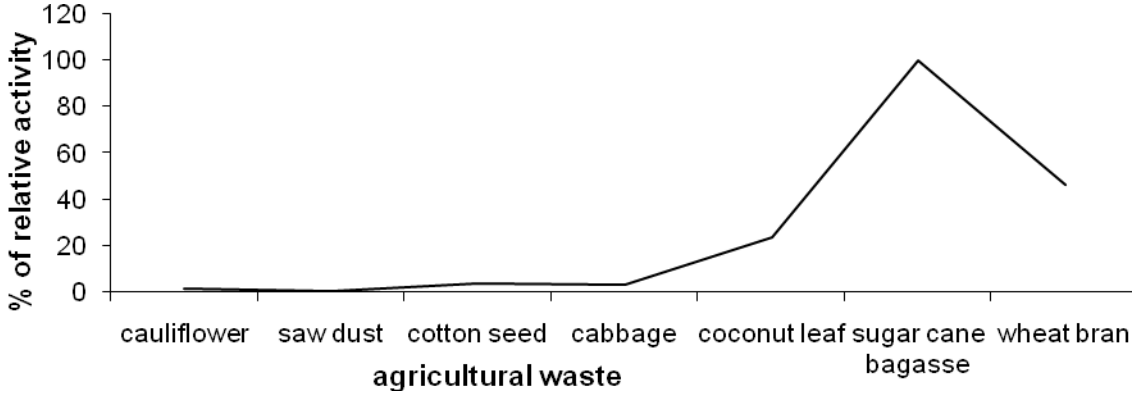

Figure-2
Screening of agricultural substrates

Enzyme production depends on various factors. The proper incubation period is most favorable parameter of solid state fermentation for producing cellulase. The result shows optimum incubation period for production of cellulose. The maximum cellulose activity of wheat bran after 3 days of fermentation was 0.23 U/g whereas for sugar bagasse it was 0.229 U/g.

Conclusion

The present work concluded that the incubation period is most important factor for growth of microorganism as well as the level of production of cellulose enzymes. The plant pathogen *M. verrucaria* is capable to producing significant yield of cellulase from wheat bran and sugar bagasse containing cellulose. Cellulase activity was increased when the incubation period increased to 3 days.

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