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Full Length Research Paper

Cellulase production from waste paper using *Trichoderma* species isolated from rhizospheric soil

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The recent interest in bioconversion of cellulosic wastes to value added chemicals has led to extensive studies on microorganisms capable of producing the enzyme. The possibility of using waste paper for the production of cellulase by *Trichoderma* species isolated from the soil was investigated using submerged fermentation technique. The fermentation temperature and pH were varied. Results indicated that maximum cellulase production of 0.18 IU/ml/min was obtained at 96 h. Maximal enzyme activity was at pH 5 when compared to the activity obtained at pH of 6.0 (0.11 IU/ml/min) and 7.0 (0.06 IU/ml/min). Incubation temperature influenced the cellulase production greatly with about 50% of the cellulase activity lost when the temperature was switched from 28 to 37°C. The cultivation and application of the organism in cellulose hydrolysis will lead to efficient conversion of cellulose materials to other important products.

Key words: Cellulose, *Trichoderma*, cellulase, submerged, fermentation, wastepaper.

INTRODUCTION

Lignocellulosic wastes are among the most plentiful complex organic carbons in form of plant biomass (Zhu et al., 2006). Lignocellulosic biomass includes but not limited to forestry, agricultural and agro-industrial wastes and is inexpensive, renewable and abundant energy sources. Such wastes include a variety of materials such as sugarcane bagasse, leaves, sawdust, brewer's spent grains, waste paper, switchgrass, and straws from cereals like rice, wheat, corn, sorghum, barley, even their husks, shells and peels, among others is made up of three components; cellulose, hemicellulose and lignin.

The cellulose portion is a linear homopolysaccharide of β-1,4 linked D-glucose residue and the hemicelluloses is an amorphous, branched structure consisting of both five- and six-carbon sugars whereas the lignin generally contains three aromatic monomers in addition to large amounts of phenolic acids (Badhan et al., 2007). The complete conversion of lignocellulosic wastes may be crucial in addressing some sustainable social development issues as its exploitation for bio-product development could help reduce the incineration practice in the third world which increases green house gases (van Steenis, 2005). The most assuring way is to change these wastes into value added products by enzymatic hydrolysis into fine chemicals.

The conversion process is two dimensional: hydrolysis of the lignocellulosic materials to fermentable sugars and subsequent fermentation of the sugars into the desired products (Zhu et al., 2006). Hydrolysis and fermentation of lignocellulosics is rather more challenging than the fermentation of sugar alone. During hydrolysis the cellulosic part of the structure is converted to fermentable sugars, and the subsequent fermentation converts the fermentable sugars to ethanol. The pre-treatment step is needed in hydrolysis to soften the biomass and to break down the cell structures to a large extent (Hamelinck et al., 2005). Pre-treatment processes are primarily chemically catalysed whereas the conversion reaction is usually aided by the cellulase enzyme system secreted by suitable microorganisms (Saber et al., 2010). Cellulases are produced by many species of bacteria and fungi, as well as by several plants. A number of cellulases

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have been identified in *Trichoderma reesei* (Foreman et al., 2003). Thus agricultural wastes and indeed all lingocellulosics can be converted into products of economic value such as ethanol, glucose, single cell protein, enzymes and organic acids (Solomon et al., 1999). Carboxymethyl cellulose which is the conventional substrate is not available in sufficient quantities and or at prices affordable to furnish the feedstock for the enzyme fermentation. Waste paper is often available in large quantities in our environment and does not find any important application commercially as they are generally disposed of by incineration, leading to the production of green house gasses that portends serious risks to the environment. With this scenario, it is essential to look for processes that permit the systematic elimination of this waste while concomitantly converting it to value added product. Hence the present study is carried out to eval-uate cellulase production from waste paper using *Trichoderma* species isolated from rhizospheric soil.

MATERIALS AND METHODS

Trichoderma harzianum medium

The *Trichoderma* selection medium (THSM) described by Askew and Laing (1993) for soil samples was adapted. The THSM consisted of a basal medium comprising (g/L) 0.8 of $MgSO_4 \cdot 7H_2O$, 3.6 of K_2HPO_4, 4.0 g of NH_4NO_3, 0.15 g of KCl, 0.6 of rose Bengal, 12 of glucose, and 20 of Agar in 950 ml of distilled water, which was autoclaved at 121°C for 15 min and then 1.0 g of chloramphenicol, 9.0 ml of streptomycin stock solution (1% wt/vol), 0.8 g of nystatin (all amounts are per liter), all in 40 ml of sterile distilled water, and the mixture was added to the cooled basal medium.

Isolation of *Trichoderma* and screening for cellulase

Rhizospheric soil samples near a decomposing wood obtained from a dump site of the Biological garden of Department of Applied Science were collected into fresh polyvinyl bags and brought into the laboratory for culturing. A homogenate of soil sample was obtained using serial dilution plate technique and aliquots inoculated directly onto the gelled THSM plates. They were incubated at ambient temperature of 28°C for a week. Spore inoculum from the growth was then re-inoculated onto a microcrystalline cellulose agar medium containing congo red indicator (Teather and Wood, 1982) and incubated at 28°C for 24 h. The colonies on the plate with surrounding clear zones are positive for cellulase activity.

Preparation of inoculum

Spores from the cellulase positive culture was sub cultured on plates of Czapek Dox agar (CDA) plates for 120 h at room temperature to obtain confluent spores for inoculation unto the fermentation medium. The spores colonies were covered with 10 mL of sterile distilled water containing 0.1% Tween 80 and the suspensions made by gently probing the surface with the tip of a sterile glass rod. The inoculum ratio was 10% v/v of the entire volume. Inoculations into the fermentation flasks containing the mash were achieved using sterile pipettes. The spore suspension was diluted to concentrations of 1.0×10^6 cells per ml.

Substrates

Waste papers were used as test substrates. They were obtained from a business center within the main campus of Kaduna Polytechnic. Sample substrates were shredded into small pieces and dried in an oven at 65°C for 24 h. The dried samples were subsequently pulverized in an electric blender (Philip) as a form of physical pretreatment. The fluffy wool-like substrate was stored in polyvinylchloride bags until use.

Chemical pretreatment of substrates

Twenty gram (20 g) of the pulverized substrate and 300 ml of (1%) NaOH solution were mixed in a 500 ml capacity conical flask and kept for 6 h for hydrolysis. The alkali treated substrate was washed in running tap water until the wash water became neutral. Excess water was removed by squeezing the substrate after which the alkali treated substrate was dried in an oven set at 65°C for 24 h.

Medium composition and fermentation

Submerged fermentation (SmF) technique using shaker at 150 rpm was adopted. The fermentation medium consisted of mineral salts and trace elements. The substrate {pretreated} was fermented in Mandel's medium as proposed by Mandels et al. (1981) with the addition of ten gram (10 g) per litre of the substrate. The medium consisted of (gL^{-1}): yeast extract, 0.2; peptone, 1; $(NH_4)_2 SO_4$, 4; KH_2PO_4, 2; Urea, 0.3; $MgSO_4 \cdot 7H_2O$, 0.3, $CaCl_2$, 0.3; $FeSO_4 \cdot 7H_2O$, 0.5; Mn $SO_4 \cdot 4H_2O$, 0.16; $ZnSO_4$, 0.14; $CoCl_2$, 2; Tween-80, 0.1% and pH adjusted using 0.05 M sodium hydroxide solution. The effect of pH on cellulase production was studied and was therefore adjusted to 5.0; 6.0 or 7.0 prior to autoclaving. The pH of the growth medium was not controlled during the course of fermentation.

Determination of cellulase activity

The cellulase activity was estimated as FPase activity units (Ghose, 1987). A Whatman number 1 filter paper was cut into a 1×5 cm strip and added to a volume of 1.5 ml of culture filtrate and 0.5 mL buffer (0.05 M citrate buffer, pH 4.5). Samples were incubated at 50°C for 1 h and the hydrolysis terminated by addition of 3 ml of dinitrosalicylic acid (DNS) solution, followed by 5 min boiling. After cooling, 20 ml of distilled water were added and the absorbance was read at 540 nm using glucose as standard. Enzyme activity was expressed as unit (UI/ml/min) per g of fermented substrate.

RESULTS AND DISCUSSION

The result of the enzyme activity per time is presented in Figure 1. The highest enzyme activity of 0.15 IU/ml/min was obtained at 96 h into the fermentation process when the pH was 5.0 and fermentation carried out at room temperature (28°C), there was a gradual rise in enzyme activity from day 1 and peaked off at day 4. Cellulase production level was elevated to the maximum at about 96 h of production. Substantial decrease in enzyme production level was detected at day five (0.06 IU/ml/min). According to Howell (1978), accumulation of cellobiose in the medium could result into end product inhibition, thus inhibiting the endoglucanase and Beta-glucosidase enzymes of the organism.

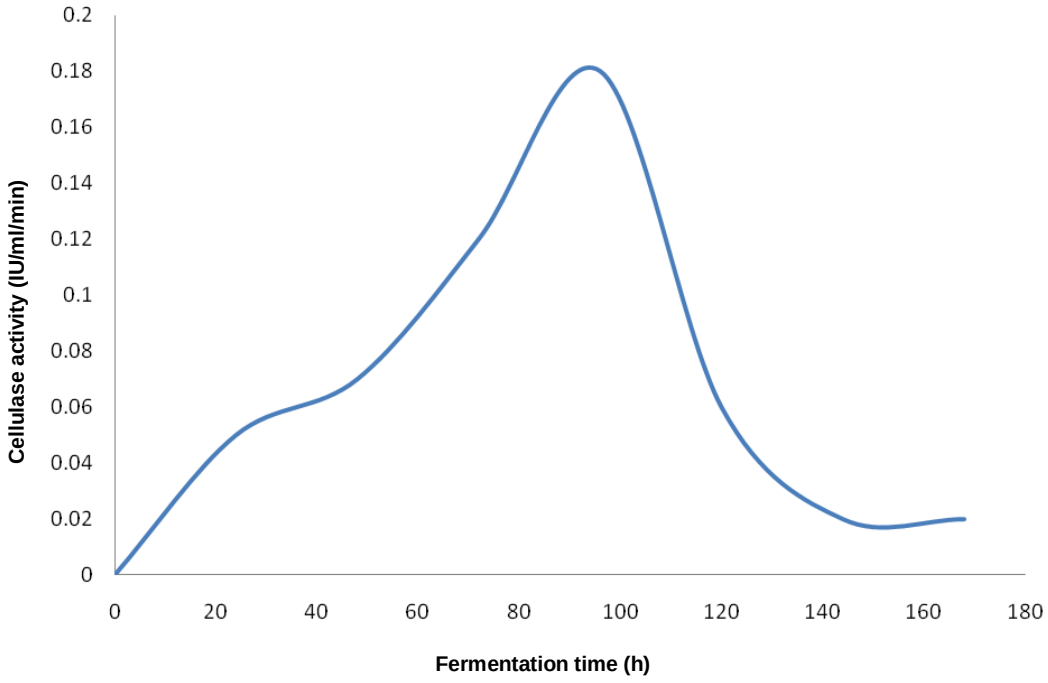

Figure 1. Cellulase activity of the *Trichoderma* species Isolate on waste paper at pH 5 and 28°C.

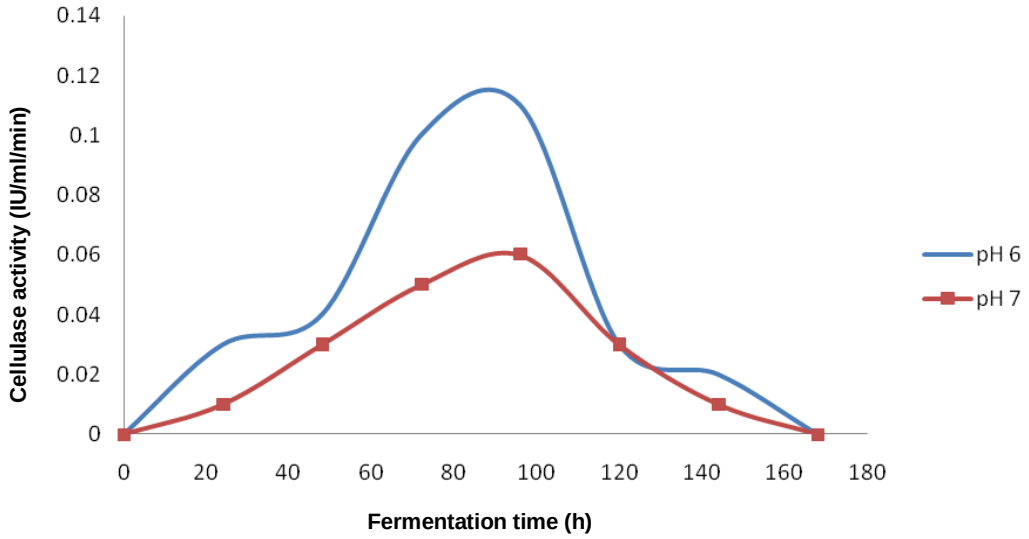

Figure 2. Effect of pH on cellulase yield from *Trichoderma* species fermented on waste paper at 28°C.

Effect of pH

The effect of pHs was studied on cellulase production level of the test organism and the result is shown in Figure 2. Two pHs were studied: 6.0 or 7.0 (apart from that of 5.0). A variation in the rate of enzyme activity was recorded for the isolate. Maximal enzyme activity was at pH 5 when compared to the activity obtained for pH of 6.0 (0.11 IU/ml/min) and 7.0 (0.06 IU/ml/min). The minimum was recorded at pH 7 even though the organism produced enzyme on all the pH used. The result clearly showed that the maximum optimal pH for cellulase production was 5. Decrease in the pH of the culture medium from optimum reduced the enzyme activity. According to Kredics et al. (2003) *Trichoderma* isolates are capable of growing at pH levels of 2.0 to 6.0.

Effect of incubation temperature

The effect of incubation temperature on the cellulase

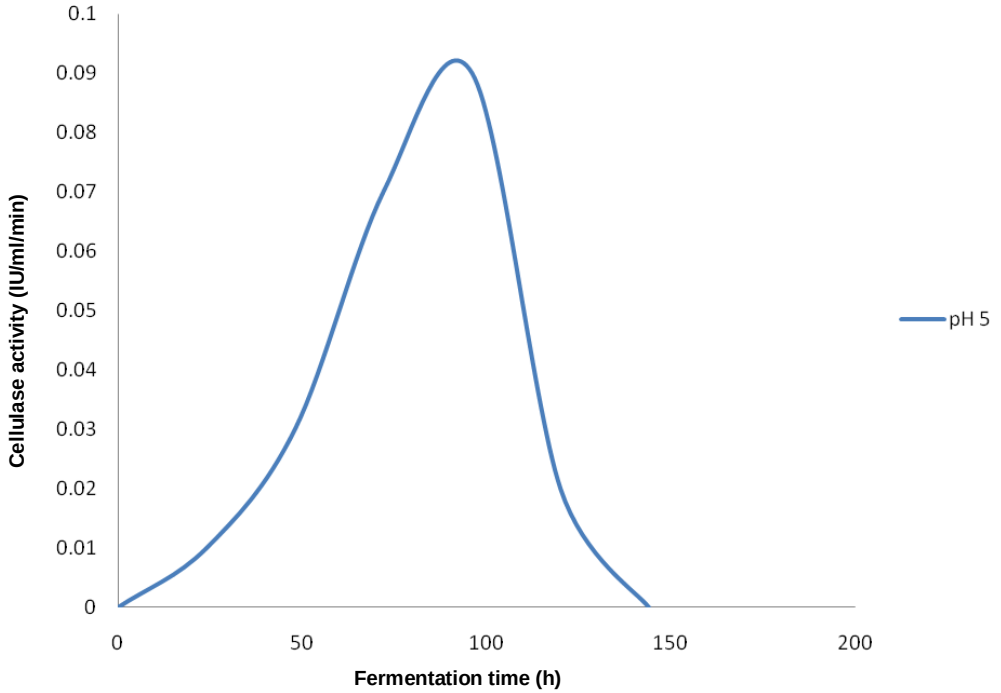

Figure 3. Effect of Incubation temperature (37°C) on cellulase yield from waste paper using *Trichoderma* species Isolate.

activity of the isolate was studied in order to determine the suitable temperature for maximal secretion of enzyme and the result is presented in Figure 3. In addition to the room temperature (28°C), incubation temperature of 37°C was studied. Optimal enzyme activity was recorded 28°C. This is to be expected because most fungal fermentations occur at room temperature. Higher temperatures may interfere with the enzyme system. It has been reported by Jayaswal et al. (2003) that temperature between 20 to 37°C is good growth of *Trichoderma* species.

Conclusion

The results proved the possibility of converting waste paper into cellulase by *Trichoderma* isolated from the soil. The alkali pre-treatment given the waste paper causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, and a decrease in crystallinity. The waste papers are mainly composed of cellulose, which must be hydrolyzed to glucose before being utilized as carbon source. Therefore, the pretreatment is important and necessary. The enzymatic degradation of cellulosic wastes by fungal enzymes has been suggested as a workable alternative for the conversion of lignocellulosics into chemicals of high value. The results showed that the maximum enzyme production from the waste papers was 0.18I U/ml/min and was optimum at 96 h which means the enzyme can be harvested at that time. Cellulase activities proportionally decreased with reduced pH and increase in temperature of incubation.

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