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Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob

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Bagasse, corncob and sawdust were used as lignocellulosic substrates for the production of cellulase enzyme using *Aspergillus flavus* after ballmilling and pretreatment with caustic soda. From the fermentation studies, sawdust gave the best result with an enzyme activity value of 0.0743IU/ml while bagasse and corncob gave 0.0573IU/ml and 0.0502IU/ml respectively. The three lignocellulosics gave their maximum enzyme activities at about the twelfth hour of cultivation, suggesting that the 12th hour is the optimum time when the enzyme may be harvested.

Key words: *Aspergillus flavus*, cellulase activity, lignocellulosics.

INTRODUCTION

Agricultural wastes and in fact all lignocellulosics can be converted into products that are of commercial interest such as ethanol, glucose, and single cell protein (Solomon et al., 1999). Cellulase enzyme has been reported (Fan et al., 1987; Wu and Lee, 1997; Solomon et al., 1999; Kansoh et al., 1999) for the bioconversion of lignocellulosics to these useful products. Solomon et al. (1990) achieved hydrolysis of sawdust using cellulase with activity of 0.0561IU/ml.

Lignocellulosics are abundant sources of carbohydrate, continually replenished by photosynthetic reduction of carbon dioxide by sunlight energy (Fan et al., 1987). Thus they are the most promising feedstock for the production of energy, food and chemical (Wu and Lee, 1997; Solomon et al., 1999). The bioconversion of cellulosic materials has been receiving attention in recent years. It is now a subject of intensive research as a contribution to the development of a large-scale conversion process beneficial to mankind (Kumakura, 1997). Such process as suggested by Fan et al. (1987) and Kumakura (1997) would help alleviate shortages of food and animal feeds, solve modern waste disposal problem, and diminish man's dependence on fossil fuels by providing a convenient and renewable source of energy in the form of glucose.

Some features of natural cellulosic materials are known to inhibit their degradation/bioconversion (Solomon et al., 1990, 1999). These are degree of crystallinity and lignification and the capillary structure of cellulose. The crystallinity and lignification limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan et al., 1987). However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported (Kansoh et al., 1999; Depaula et al., 1999; Solomon et al., 1999; Kumakura, 1997; Wu and Lee, 1997). Pre-treatment of cellulose opens up the structure and removes secondary interaction between glucose chains (Tang et al., 1996; Fan et al., 1987). Solomon et al. (1999) produced cellulase of 0.056425 IU/ml from the growth of *Aspergillus flavus* on bagasse pre-treated with using ballmilling and caustic soda.

Since the production of cellulase enzyme is a major factor in the hydrolysis of cellulosic materials, it is

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important to make the process economically viable. Although much work has been done on the production of cellulase from lignocellulosics (Solomon et al., 1999; Depaula et al., 1999; Kansoh et al., 1999), emphasis has been placed much on bagasse. This work focused at improving its yield by using various sources of lignocellulosic namely; sawdust, corncob and bagasse inclusive. Thus assessing the effect of the various lignocellulosic source on the yield of cellulase by the growth of *A. flavus*.

MATERIALS AND METHODS

Lignocellulosic sources and pre-treatment

The substrates used for this work are bagasse, sawdust and corncob; they are cheap and readily available sources of lignocellulosics. The bagasse was collected from Nigeria Sugar Company, Bacita, Kwara State, Nigeria. The corncob was obtained from Obafemi Awolowo University Research Farm and the sawdust from a sawmill in Ile-Ife, Nigeria. The substrates were sundried for two days so as to reduce the moisture content and make them more susceptible to ballmilling.

The substrates were ballmilled at about 60 rpm for 48 h after which the ballmilled substrates were individually screen analysed in the Endecott test sieve shaker and each sample was made to pass through a 0.5 mm screen. The samples were then soaked in 1% (w/v) sodium hydroxide solution at a ratio of 1:10 (substrate:solution) (Gharpuray et al., 1983; Solomon et al., 1999) for 2 h at room temperature after which it was washed free of the chemicals and autoclaved at 121^{o}C (15 psig steam) for 1 h. The treated substrate was then filtered and washed successively with distilled water until the wash water was neutral.

Inoculum Preparation

A pure culture of *A. flavus* Linn Isolate NSPR 101 was provided by the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. This was used throughout this study. The organism was maintained as direct stock culture from which inocula were prepared. It was grown on malt extract agar slant at 30^{o}C for 5 days and stored at 4^{o}C with regular subculturing. 200 ml of the optimised medium (Solomon et al., 1999) of each sample with *A. flavus* from a 4 day culture was used as inoculum prepared in a 250 ml. The inoculum was shaken continuously on an environment-controlled incubator shaker (New Brunswick Scientific Co., USA) at 200 rpm and 35^{o}C for 24 h before it was used for the fermentation process.

Fermentation Experiment

Different fermentation runs were carried out on the batch fermentor unit of the double-unit Microferm Fermentor. Two litre of the optimised medium containing: 30 g/l pre-treated substrate, 0.3 g/l of L-glutamic acid, 1.4 g/l NH_4NO_3, 0.2% of Tween 40, 2.0 g/l of KH_2PO4, 0.3 g/l of $CaCl_2$, 0.3 g/l of $MgSO_4$, 0.75 g/l protease peptone, 5.0 mg/l of $FeSO_4.7H_2O$, 1.6 mg/l of $MnSO_4$ .H_2 O, 1.4 mg/l of $ZnSO_4.7H_2O$ and 2.0 mg/l of $CoCl_2$ were mixed in a 7½ litre fermentation vessel made of borosilicate glass and sterilised with its accessories at 121^{o}C for 15 min.

After sterilisation, the vessel was cooled to room temperature and the impeller shaft was coupled and filters were plugged into their receptacles. The inoculum was introduced aseptically and fermentation proceeded at agitation of rate of 200 rpm and aeration maintained at 1.0 vvm. The fermentation was maintained at 35^{o}C for 52 h, sample of the medium was withdrawn every 4 h and the supernatant was analysed for reducing sugar and enzyme activity after centrifugation.

Determination of reducing sugars and cellulase activity

The total amount of reducing sugars (expressed as equivalent glucose) in 1.0 ml supernatant was determined by the modified dinitrosalicyclic acid (DNS) method of Miller (1959) as applied by Solomon et al. (1999). Cellulase activity was determined as a Filter Paper Activity (FPA) by the method of Ghose (1987) also applied by Solomon et al. (1999).

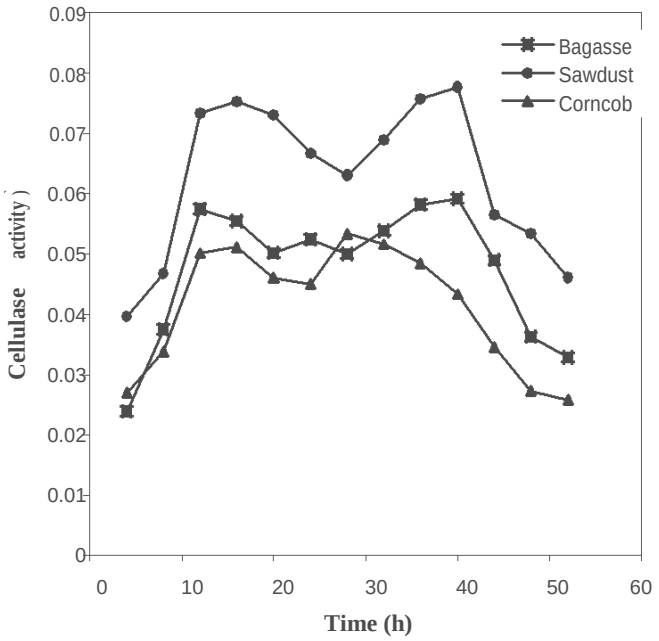

Figure 1. Substrate effect on A. flavus cellulase activity grown in optimised medium at 200 rpm agitation and 1.00 vvm aeration.

RESULTS AND DISCUSSION

Figure 1 shows the plot of enzyme activities in the various substrates (measured as filter paper activity) at the time interval of 4 h during the fermentation studies of A. flavus. The figure shows the effect of the various lignocellulosics on the production of cellulase enzyme produced. The results presented here show the extent of production (yield) of cellulase enzyme for a period of 52 h measured as enzyme activity and also the production rate of the enzyme measured as the ratio of the yield to

time. Cellulase activity increased to the maximum at about 12 h of production for all the lignocellulosic materials used. The stability of the cellulase spanned from 12 to 40 h except in corncob where there was a sluggish decrease from 28 to 40 h before the eventual decrease in the activity. Solomon et al. (1999) have previously reported that the enzyme could be harvested at about 12th h, when the activity is highest.

A. flavus grown on sawdust gave the highest cellulase activity of 0.0743 IU/ml, while bagasse and corncob gave 0.0573 and 0.0502 IU/ml, respectively. The depression in cellulase activity between 20th and 30th h, common to all three substrates, may be due to cumulative effect of cellobiose, a dimer of glucose which is known to inhibit both endoglucanase and -glucosidase (Howell, 1978). Hatakka (1983) also suggested that delignification produces aromatic water-soluble products which can repress the cellulolytic action of the enzyme.

The value obtained when the lignocellulosic was bagasse compares favourably with 0.056425 IU/ml reported by Solomon et al. (1999) using the same operating conditions. The estimated production rates are 0.00478, 0.00619 and 0.00418 IU/(ml-h) for bagasse, sawdust and corncob, respectively. The highest cellulase productivity with sawdust may be due to its very high percentage of cellulose which is the major component of cell walls of wood.

In conclusion, *A. flavus* is capable of producing cellulase from sawdust and corncob in addition to bagasse that was previously used. Cellulase enzyme produced from sawdust corncob and bagasse could be harvested at 12 hours of production, the time at which the activity is highest. Sawdust is most suitable for cellulase production compared to bagasse and corncob as it gave the highest yield of the enzyme.

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