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Production and activity of cellulase from *Aspergillus niger* using rice bran and orange peel as substrates

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ABSTRACT

The production and factors controlling the activity of celluloytic enzyme secreted by *Aspergillus niger* in submerged culture with rice bran, orange peel, and Carboxymethylcellulose (control) as substrate was studied. The effects of pH, temperature and substrate concentrations on the enzyme activity were also studied. Optimal cellulase secretion was achieved in 94 hours of growth in rice bran, and Carboxymethylcellulose with cellulase activities as 16.63µmol/ml, and 3.61µmol/min respectively. Within the growth period of 48 hours, cellulase activity of 14.30 mol/min was observed in orange peel. Substrate concentration of 6 and 8% w/v favoured the cellulase activity. While pHs 3 and 4 produced optimal enzyme secretion. Also temperatures of 45ºC and 50ºC favoured cellulase production.

INTRODUCTION

Enzymes of commercial or industrial importance are obtained from three main sources namely plants, animals and microorganisms. In the past, plants and animals served as main source of enzymes but today microbial sources of enzyme are becoming more popular for obvious reasons (Abu *et al.,* 2000). In order to obtain even a small quantity of plant enzymes, a large amount of plant materials has to be used and this renders large scale production of plant enzymes uneconomical, especially if the plant has some economic values or uses. Also difficulties are encountered in the extraction of the enzyme from plants (Howard *et al*, 2003). In the case of animal source, enzymes obtained from them are usually by-products of the meat industry and hence the supply can be limiting. Also other valuable products may be needed from the same organs used for enzyme production; such competition will further reduce the amount of materials from which such enzymes can be extracted. On the other hand, microbial enzymes are not subject to any of the problems of plant and animal enzyme (Emmanuel *et al*., 2007). In addition, the number of microbial enzymes is almost limitless, while the number, mode and amount to be produced at a time can be manipulated by the producers. Furthermore, enzymes of commercial values are extracellular in nature and are thus released into the cultured medium of the microorganisms and can be obtained by filtration and centrifugation rather than the vigorous methods of extraction at the end of the fermentation (Abu *et al.,* 2000).

Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria and protozoan that catalyzed the cellulolysis (or hydrolysis of cellulose). Although there are also cellulose producing plants and animals, a large number of microorganism are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell free enzyme capable of degrading cellulose *in vitro.* Fungi carry out extracellular digestion and secrete digestive enzyme into their substrates and absorb only digested food into their hyphae as such, they produce cell free enzyme. Fungi are the main cellulase producing microorganism in which *Aspergillus sp*. are known to hydrolyse both soluble and insoluble cellulose (Sridevi *et al.*, 2007).

Cellulase is used for commercial food processing in coffee. It performs hydrolysis of cellulose during drying of coffee beans. Furthermore, cellulase is widely used in textile industry and in laundry detergents. Cellulase has also been used in the pulp and paper industry for various purposes. They are even used in pharmaceutical applications. Cellulase is used in the fermentation of biomass into biofuels, although this process is relatively experimental (Okafor *et al.,* 2007). Cellulase is used as a treatment for phytobezoars, a form of cellulose bezoar found in the human stomach.

Since the production of cellulase enzyme is a major factor in hydrolysis of cellulosic material, it is important to make the process economically viable; this study therefore investigated the bioconversion of agricultural waste like rice bran and orange peel (which could cause pollution to the environment) into a more useful product (cellulase) using *Aspergillus niger*.

MATERIALS AND METHODS

Sources of agricultural waste and pre-treatment

The substrates used for this work were rice bran and orange peels. The rice bran was obtained from Ipata Market, while orange peels were obtained from Ago Market in Ilorin, Kwara State. The substrates were washed thoroughly with water to remove surface dirt, and oven dried at 70^0C for 2 hours. The dried samples were broken into pieces form with the aid of mortar and pestle in preparation for alkaline and steam treatment (Ali *et al*., 1991). One gram each of the broken samples was measure into separate conical flasks containing 20ml of 5% NaOH solution. This was autoclaved at 121^oC for 1 hour to free cellulose of lignin hold. The NaOH solution was drained off by sieving through a muslin sieve. Samples were rinsed several times with distilled water, neutralized with 0.1 M HCL and finally washed with distilled water. The pre-treatment samples were dried in oven at 70^oC for 24 hours and further broken to powder form in an electric blender.

Preparation of Inoculums

The microorganism *Aspegillus niger* was obtained from International Institute of Tropical Agriculture (IITA), Ibadan and maintained at 4^0C on Potato Dextrose Agar (PDA) slants. It was later sub-cultured on fresh sterile carboxyl methyl

cellulose mineral salt agar slant; it was maintained at 4^0C and used throughout the experiment.

Media preparation for enzyme production

The basal medium comprised of (per litre of distilled water); KH_2P0_4, 10.0g; $(NH_4)_2$ SO_4, 10.5g; $MgSO_4$. $7H_20$, 0.3g; $CaCl_2$, 0.5g; $FeSO_4.7H_2O$, 0.013g; $MnS0_4.7H_20$, 0.04g; $ZnSO_4$. $7H_20$, 0.004g; C_OCl_2. $6H_20$, 0.0067g; yeast extracts, 0.5; into separate 250 ml conical flask containing 100 ml of the basal medium was added 40g each of the treated carbon source (orange peel and rice bran) and 20g of Carboxymethylcellulose (for control). The pH of the media was then adjusted to 5. Media were sterilized in an autoclave at 121^{o}C for 15 minute. (Milala *et al.*, 2005).

Fermentation

Media were inoculated by scooping mycelia from slant culture with the aid of a wire loop and incubated at 30^0C in an orbital shaker (Gallenhamp, England) at 100 rpm. Samples of culture fluids obtained with aid of sterile pipette every 24 hours for 192 hours. These were centrifuged at 3,000 rpm for 15minute and the supernatant analysed as the crude enzyme.

Assay of cellulase (EC 2.3.1.4) Activity

Cellulase activity was determined colorimetrically by measuring the increase in reducing sugar group by the hydrolysis of Carboxymethylcellulose (CMC) Substrate (Ali *et al*., 1991). The cultured sample was centrifuged at 3,000 rpm for 15 min, to remove the mycelia and supernatant was used for assaying enzymatic activity.

Determination of time course for enzyme production

The optimum time management for enzyme production was determined by assaying samples collected at 24 hours interval for cellulase activity on enzyme production.

Effect of varying substrate concentration

The optimal concentration of the carbon source for enzyme production was determined by measuring the activity of cellulase produced when the fungus was grown in sterile medium contain the carbon source at 2, 4, 6 and 8% w/v.

Effect of varying pH

The optimal pH for enzyme production was determined by growing the fungus in sterile media with pH adjusted to 1, 3, 4 and 6 with HCl and NaOH.

Effect of varying temperature

The optimal temperature for enzyme production was determined by growing the fungus in sterile media incubated at temperature of 35, 40, 45 and 50^{o}C. Cellulase activity of the enzyme were then determined.

RESULTS

Figure 1 showed cellulase enzyme activities for a period of 192 hours and also the production rate of the enzyme measured as ratio of yield to time. Enzyme activities were maximal at 96 hours for rice bran and for Carboxymethylcellulose (control) it was 16.63µmol/min and 3.61µmol/min respectively while at 48 hours orange peel has a value of 14.30µmol/min. All the substrates had a

decrease in the enzyme activity after 96hours for media containing rice bran, and Carboxymethylcellulose (control) except for media with orange peel that have their decline after 48hours of fermentation period. Rice bran has the highest cellulase activity than Carboxymethylcellulose (control).

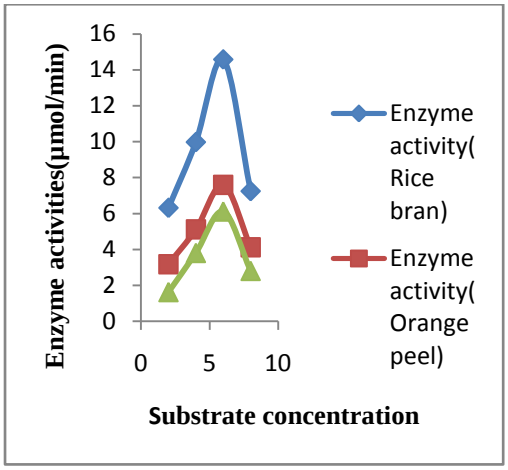

Fig. 1: **Time course for cellulase enzyme production by the organism *Aspergillus niger***

Substrate concentrations were varied as 2%, 4%, 6% and 8% w/v. Enzyme activity increased for the substrates concentrations of 2%, 4% to 6% as presented in figure 2.There was maximum cellulase activity for all the substrate used at 6%. Media containing rice bran, orange peel, and CMC (control) have their maximum cellulase activities at substrate concentration of 6% with values of 14.58µmol/min, 7.60 µmol/min and 6.11µmol/min, respectively. Rice bran has highest value while CMC (control) has the least value. However concentration above 6%, there was a drop in enzyme activity.

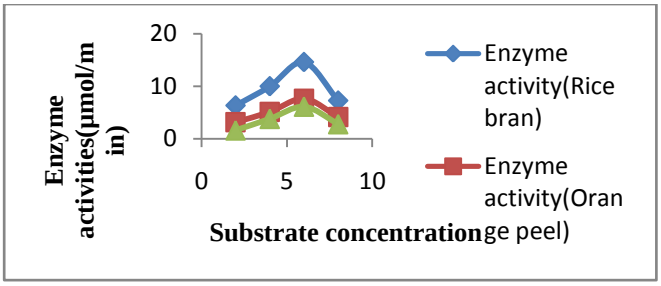

Fig.2: **Effect of substrate concentration on the production of cellulase enzyme by *Aspergilius niger***

The effect of varying pH values of 1, 3, 4, and 6 of media containing the different agricultural wastes as substrates were presented in figure 3. Media cultured with orange peel, and CMC (control) at pH value of 4 gave maximum cellulase activities of 9.07µmol/min and 12.41µmol/min respectively, while media with rice bran gave the maximum cellulase activity of 14.33µmol/min at pH value of 3. Media cultured with rice bran has highest activity at pH value of 3 than orange peel.

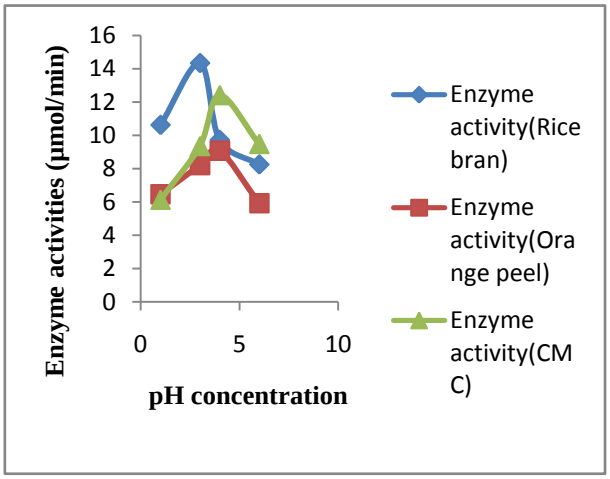

Fig.3: **Effect of pH on the production of enzyme by *Aspergillus niger***

The effect of temperature on cellulase activities increased from 35°C to 50°C but the highest cellulase activity was at temperature of 45°C as presented in figure 4. Rice bran produced maximum cellulase activity at temperature of 45°C with value of 20.35µmol/min, CMC produced maximum cellulase activity at 40°C with values of 12.13µmol/min while orange peel produced maximum cellulase activity at temperature of 50°C with values of 15.03µmol/min. Rice bran produced the highest cellulase activity while CMC has the least value of cellulase activity.

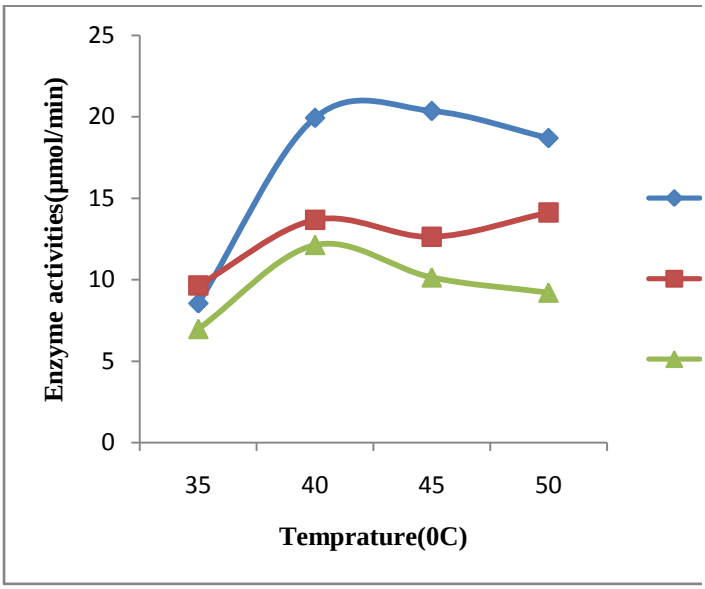

Fig. 4: **Effect of temperature on the production of cellulase enzyme by *Aspergillus niger***

DISCUSSION

The results showed that *A. niger* produced cellulase enzyme (E.C.3.2.1.4) when cultured on mineral salt media containing rice bran or orange peel as sole carbon source. Extracellular protein with significant cellulase activity was obtained from the cultures of all the different carbon sources (figure 1). Most members of the *A. niger* group are notable producers of extracellular enzyme such as cellulase (Solomon *et al.,* 1999). The highest level of cellulase activity was obtained with rice bran at 96 hours.This is in line with finding of Ali *et al.,* who reported that enzyme could be harvested at about 72 hours of fermentation. Carboxymethylcellulose substrate (control) gave the least cellulase activity at 96hrs while orange peel substrate produced it highest activity at 48 hours. The decrease activity in rice bran, orange peel, carboxymethylcellulose, after a fermentation period of their highest activities may be attributed to cumulative effect of cellobiose (Lee *et al.,* 2007). Cellobiose, a dimer of glucose is known to inhibit both endoglucanase and glucosidase. It may also suggest that delignification produce aromatic water soluble products that repress the cellulolytic action of the enzyme. This is supported by the finding of Emmanuel *et al.,* (1991) who reported the, inhibitory effect of accumulated cellobiose and cellodextrin of low degree of polymerization. The decrease may also be due to depletion of the other nutrients (Mineral - Salt) other than the energy source.

The increase in enzyme activity in rice bran as the substrate from 2% to 6%, suggests the ability of rice bran to produce more of the cellulase at optimal substrate concentration of 6%.

At pH value of 3, rice bran were found to have its highest cellulase activities while at pH value of 4 carboxymethylcellulose and orange

peel have their highest activities. The instability of these enzymes at very low or very high pH (values) is due to the fact that they are proteins which are generally denatured at extreme pH values. This is in agreement with the work of Ali *et al.,* (1991) in which pH of 3 and 4 were reported as favouring high yield of cellulase enzyme.

At temperature of 40^{o}C carboxymethylcellulose were found to support highest cellulase activity while at 45^{o}C rice bran support highest cellulase activity and at 50^{o}C orange peel has its highest activity. This is in line with the finding of Ogundero, 1982, who reported that culture cellulase of *A.niger* were found to be more active at temperature of 45-50^{o}C.

CONCLUSION

Rice bran and orange peel have been used in production of cellulase. Rice bran and orange peel supported the production of cellulase by *Aspergillus niger*. The results highlight the potentials of the substrates as possible raw materials for cellulase production using *Aspergilius niger*, with rice bran being a more suitable substrate because of its ability to support production of more cellulase activity than the other substrate used.

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