African Journal of Biotechnology Vol. 3 (8), pp. 395-398, August, 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont.) Singer

Shide EG[1], Wuyep PA[2]* and Nok AJ[3]

[1]Department of Chemical Engineering, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

Accepted 30 July, 2004.

***Lentinus squarrosulus* (Mont.) Singer, a basidiomycete also known as a white rot fungi, was immobilized on sodium alginate and tested for the effectiveness to degrade wood sawdust (WSD). Untreated and 0.1 M HCl-pretreated WSD samples were separately reacted in a micro-carrier bioreactor (μCBR) and the extent of degradation to form protein, glucose and ethanol was investigated. Pretreatment enhanced the production of both proteins and ethanol by average value of 72.0% over untreated WSD samples, after hydraulic retention time of 72 h. The maximum production of protein observed was 0.94 mg/ml-reaction volume and that of ethanol was 6.6 mg/ml-reaction volume, whereas glucose concentration fluctuated due to interconversion to ethanol. This report shows that *L. squarrosulus* (Mont.) Singer have the potentials of degrading WSD samples to important chemical compounds that are not hazardous to the environment.**

Key words: *Lentinus squarrosulus*, wood sawdust, sodium alginate, bioreactor, ethanol.

INTRODUCTION

Wood is an essential material for man. It is a material source for energy and constructional works. Wood debris, byproduct of wood processing, pollutes the environment even though these debris are materials suited for biodegradation (Williams, 2001). Wastes and their disposal have become enough substances of environmental concern worldwide especially when these wastes are biodegradable to useful goods and services.

Three major wastes management routes have been identified (Williams, 2001) namely, sewage disposal, composing and landfill and bioremediation. Amongst these, the sewage disposal provides opportunity for any possible recovery of useful product after biodegradation. Cultivation of enzymes for degradation of lignocellulosic materials has been reported (Banjo and Kuboye, 2000; Wuyep et al., 2003). The negative concerns other people have regarding wastes differ from scientific views; wastes are no longer scraps instead are unused resources. DuPont, a company with environmental wastes management at heart, is of the view that 'waste manufacturing may be a product looking for a market' (Keith, 1994) and saved on average $50,000,000.00 per year at the beginning of 1985 through early 1990 because of its waste management activities.

The use of biological means have greater advantages over the use of chemicals for degradation because biotechnological synthesized products are less toxic and environmentally friend (Liu et al., 1998). Also the use of immobilized cell eases process design because cells attached to large particles or on surfaces are easily separated from product streams. The products quality is assured since cells are prevented from diffusing into product streams and cell washout in continuous operation is hindered (James, 1992). The current effort is aimed at

*Corresponding author. E-mail: egshide@Yahoo.com.

transforming wood sawdust (WSD) into glucose, protein and ethanol that are of benefits.

MATERIALS AND METHOD

Fungal cultivation

White-Rot-Fungi, *Lentinus squarrosulus* (a basidiomycetes), was cultivated by Anderson method (Anderson et al., 1973). The mushroom gill were replaced on sterile glass slide in a sterile petri dish and covered to prevent air current and the resultant air borne contamination. The slides bearing the spores were then transferred into sterile envelopes and stored in refrigerators at 4°C for future use. The procedure for obtaining the culture of *L. squarrosulus* culture was as previously described(Wuyep et al., 2003), and further sub-cultured on a medium consisting of the following (%,w/v): malt extract (3.36), KH_2PO_4 (0.001), NaH_2PO_4 (0.002), $MgSO_4.7H_2O$ (0.005), dimethyl succinate (0.0015), thiamine hydrochloride (1.0), $FeSO_4.7H_2O$ (1.0), $ZnSO_4.7H_2O$ (1.0), $CuSO_4.5H_2O$ ($_2$.0), H_3BO_3 (2.0) and distilled H_2O (10.0).

Cell immobilization

The method adopted for the cell immobilization is according to the one described by James (1992) with slight modification. The cells of the exponentially growing mycelia of the culture were harvested aseptically using a sharp knife into a one-liter capacity blender (Master Chef 650, UK). The harvested cells were then homogenised in the blender and about 17.5 ml of the homogenate were measured into 250 ml conical flask containing 87.5 ml distilled water and mixed properly to ensure homogeneity. This was allowed to settle after 10 minutes and separated. The supernatant (concentrated cells) was stored at 20°C.

Exactly 3.063 g of sodium alginate (Hopkin & Williams limited, England) was weighed into the concentrated cells. The mixture was subsequently pumped through a sterilized silicone tube (1.6 mm ID) drop-wise into a flask containing sterilized 70 ml of 0.12 M $CaCl_2$ (May & Baker Limited, England). The reaction, which was almost instantaneous, was allowed for retention time of 1h for complete precipitation that formed spherical beads of diameter 3.75 - 4.5 mm. The immobilized cells were removed and stored in distilled water at 4°C.

Preparation of wood sawdust solution

Wood sawdust was reduced to particle size 152 μm using a grinding machine (SK-1 Retsch KG, West Germany). About 6.0 g sawdust was transferred into 500 ml micro-carrier bioreactor (μCBR, 1965-00500, M3622129, Belco, U.S.A.) and made to the level with distilled water. The pH of the solution was adjusted to 6.8 using 0.1 M NaOH. The solution was autoclaved at 80°C for 10 min and applied as untreated WSD sample. The same procedure was followed for preparing 0.1M HCl-pretreated WDS sample except for the acidification with 10 ml 0.1HCl. The autoclaved solutions were maintained at 29°C before use.

Degradation of sawdust solution

About 400 beads of the immobilized cells were added to each of the autoclaved solutions with a magnetic stirrer (5 mm) that induced stirring effect of about 150 rpm at the specified temperature. Aliquot of the solution were taken first at hourly intervals for five hours, then continued daily for three days and the results for each sample were recorded for the contents of protein, glucose and ethanol.

Glucose and Protein Determination

Glucose was determined using dinitrosalicylate (DNS) reagent. Standard glucose solution was prepared by dissolving 10 g 3,5-dinitrosalicylate in 200 ml 2 M-NaOH and mixed with solution of 300 g Rochele salt in 500 ml water. This mixture was then diluted to 1 L to contain 2 g crude sugar per liter (5 mM glucose solution). Different dilutions of the standard DNS reagent were made and absorbances recorded. A standard curve for the determination of glucose was constructed using the results. 2 ml of test solution were measured into a test tube, 0.5 ml distilled water and 2 ml DNS were added, mixed and place on water bath at 90°C for 5 min. The content was then diluted to 20 ml with water and cooled to investigation temperature and the absorbance determined. This procedure was repeated twice and the average value of the three absorbances was determined. Using the average value of the absorbances, the concentration of the test sample was determined from the standard curve.

Protein was determined using Biuret reagent. Standard albumin solution containing 10 mg standard albumin per ml-solution was prepared and fractions of 0.2, 0.4, 0.7 and 1.0 ml were measured out into four different test tubes. Distilled water was added to the first three test tubes to make up 1.0 ml solution. 4 ml of Biuret reagent were added to each of the content of the test tubes and cautiously shaken to homogenise and left for 30 min at 29°C. The averages of three absorbances measured using a spectrophotometer (Pye Unicam, Model SP192) at 540 nm, each of the test tubes, were recorded from which the standard curve was obtained. The procedure was repeated using 1.0 ml of the test solution and corresponding concentration of protein determined from standard curve.

Ethanol Determination

Concentrated ethanol (92% v/v) was diluted to 2.0, 4.0, 6.0 and 8.0 and the absorbencies measured using a spectrophotometer (Pye Unicam, Model SP192) at of 540 nm. The standard curve was obtained from the plot of these values. The absorbencies of aliquot samples were determined and concentration found from the standard curve and appropriate conversion made and recorded.

RESULTS AND DISCUSSION

Wood is employed in several sectors including construction works, pulp and paper production, manure in the agricultural sector, and fuel in the energy sector. WSD or wood is composed essentially of moisture, cellulose, resin and wax, water-soluble and lignin at varying percentages. WSD, as a lignocellulosic material can undergo enzymatic degradation to produce protein, glucose, and subsequently ethanol, without loosing any other component that is nonbiodegradable. Ethanol is an important industrial solvent as well as a liquid fuel that has a higher calorific value (29.8 MJ kg^{-1}) than WSD, and it is usually wasted along with other products on burning or disposing WSD.

Table 1 shows progressive production of protein, glucose and ethanol, which are all of economic value

Table 1. Analysis of untreated wood sawdust degradation.

Time (h)	Protein (mg/ml)	Glucose (mg/ml)	Ethanol (mg/ml)
0	0	0	0
1	0.32	0.41	1.37
2	0.36	0.43	1.87
3	0.40	0.45	2.10
4	0.44	0.48	2.30
5	0.54	0.37	2.50
24	0.58	0.31	3.34
48	0.64	0.16	3.50
72	0.55	0.07	3.87

Table 2. Analysis of 0.1M HCl-pretreated wood sawdust degradation.

Time (h)	Protein (mg/ml)	Glucose (mg/ml)	Ethanol (mg/ml)
0	0	0	0
1	0.34	0.46	1.49
2	0.39	0.48	2.03
3	0.45	0.50	2.35
4	0.56	0.59	2.85
5	0.65	0.44	3.00
24	0.67	0.36	3.86
48	0.87	0.20	4.74
72	0.94	0.12	6.66

using untreated WSD. With increase in hydraulic retention time, production of protein and ethanol increases. However, glucose production fluctuated due to its interconversion to ethanol. After 24 h retention time, the rate of glucose production reduces, as also have been observed by Godliving and Yoshitoshi (2002,). Also the depletion of carbon and nitrogen source at the upper part of the reactor caused starvation (Dosoretz et al., 1990). The production of amylase is evident since *L. squarrosulus* has potentials of degrading sawdust into ethanol. Without this enzyme, production of glucose and subsequently ethanol cannot be achieved.

The results of Table 2 show faster metabolic activities. This is in agreement with the findings of Wiseman and Gould (1970) as well as Ryu and Lee (1983) on the effect of pretreatment of lignocellulosic material prior to degradation The use of acid pretreated lignocellulosic material in this work enhances the production of protein from a maximum of 0.55 to 0.94 mg/ml-solution mixture representing a lift of 71.0% which also leads a corresponding lift of 72.1% for ethanol production. This enhancement is due to the combined effect of chemical and biochemical attacks on the lignin (which cements cellulosics fibers together) and the highly ordered crystalline structure of the cellulose. There is also an advantage in the use μCBR because it prevents deactivating immobilized enzymes from interfacial forces (that can cause folding and rapture) by subjecting them to shear stresses. The reduction in glucose production can also be attributed to its interconversion to ethanol on fermentation (Clausen and Gaddy, 1980). Our observation on the use of pretreated WSD samples is also similar to those of Kelsey and Shafizadeh (1980), Deeble and Lee (1985), and Jones and Lee (1988).

The use of immobilized *L. squarrosulus* improve the degradation of WSD can be improved when choice environmental conditions of substrates concentration, pH, temperature and reactor size are used. Stirring at about 120 rpm which provides moderate shearing stress may not have vulgar impact on the immobilized beads to cause rupturing or denaturalization of enzymes. However, where total impact may be envisaged (particularly in fluidized beds), flow rate of fluidized mixture will be a parameter of interest to control jarring impacts of beads on the walls of the column. The most interesting part of this report is the ability to produce a biological chemical that is able to enhance degradation of lignocellulosic materials. With further application of pretreated test (WSD) samples, the retention time of the reaction is reduced and the rate of production of desire material increased. Our results also confirm that some of the environmental costs in terms of waste production are misplaced valuable raw materials awaiting prospective investors.

REFERNCES

Anderson NA, Wang SS, Schwandt JW (1973). The pleurotus ostreatus sapidus species complex. Mycologia 6:28-35.

Banjo NO, Kuboye AO (2000). Comparion of the effectiveness of some common Agro-industrial wastes in growing three tropical edible mushrooms. Proceedings of the Internal Conference on Biotechnology: Commercialization and Food Saftly, Nigeria, pp. 161-168.

Bonnarme P, Jeffries TW (1998). Mn(II) regulation of lignin peroxidase and managanes-dependent peroxidase from lignin-degrading white-rot-fungi. Appl. Environ. Microbiol. 56:210-217.

Clasen EC and Gaddy JL (1980). The Advantages of Immobilized cell Reactors in Fermentation Process. Fayettevile, Arkansas 72701, U.S.A.

Deeble MF, Lee JM (1985). Enzymatic hydrolysis of cellulosic substances in an attrition bioreactor. Biotechnol. Bioengr. Symp. 15:277-293.

Dosoretz GG, Chen HC, Greth Lein HE (1990). Effect of Environmental conditions on extracellular protease activity in lignohytic cultures of *Phanerochacte chrysosporium*. Applied Environmental Microbiology 56:395-400.

Godliving M, Yoshitoshi (2002). Continous production of lignin-degrading enzymes by Bjer Kandera adusta immobilized on polyurethane foam. Biotechnol. Lett. 24:1743-1747.

James ML (1992). Biochemical Engineering. Prentice Hall, New Jersey, pp. 101-137.

Jones EO, Lee JM (1988). Kinectic analysis of bioconversion of cellucose in attrition bioreactor, Biotechnol. Bioengr. 31:35-40.

Keith DD (1994). Enviro-Mangement: How Smart companies turn environmental costs into profit Prentice-Hall, USA, pp. 1-18.

Kelsey RG, Shafizadeh F (1980). Enhancement of cellulose accessibility and enzymatic hydrolysis by simultaneous wet milling. Biotechnol Bioengr. 22:1025-1036.

Liu CZ, Wang YC, Ouyang F, Ye HC, Li GF (1998). Production of artemisimin by hairy root cultures of *Artemisia annua* L in bioreactor. Biotechnol. Lett. 20(3):265-268.

Ryu DDY Lee SB Tassinari T and Macy C (1982). Effect of compression milling on cellulose structure and on enzymatic hydrolysin kinetics Biotechnology Bioengineering. 26: 147-1067.

Williams B (2001). Biotechnology; from A to Z, 2nd edition, Oxford Press, New York, pp. 384-385.

Wiseman A, Gould BJ (1970). Enzymes, their nature and role. Hutchinson Educational limited, London, UK, pp. 70-75.

Wuyep PA, Khan AU, Nok AJ (2003). Production and regulation of lignin degrading enzymes from *Lentinus squarrosulus* (mont) Singer and *Psathyrella atroumbonata* Pengler. Afr. J. Biotechnol. 2(11): 444-447.