J. Agric. Sci. Mansoura Univ., 25 (7): 4777 - 4783, 2000.

UTILIZATION OF COTTON STALKS

Sekina A. Mohamed
Soils, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

Three different methods has been used for the hydrolysis of the cotton stalks to reducing sugar and valuable products. These methods involves: (1) Biological degradation by cultivation of *Aspergillus niger* on cotton stalks in simple media. The bio-products of *A. niger* growth in cultures filtrate were evaluated after 6 days of incubation with shaking (~120 rmp) at 30^0C. (2) Dual-step acid hydrolysis (H_2SO_4 72%, 4%, v/v) at different time and temperature of hydrolysis.(3)- Enzymatic hydrolysis (two sources of cellulases, separately) for 3 days at 37^0C and pH 4.6. In biological degradation, the highest bioproducts in culture filtrate (1.72 mg/ml reducing sugar; 5.01 and 1.27 U/ml of $\propto$ - amylase and amyloglucosidase; 5.92 and 9.31 mg sugar / ml / h of C_x and X enzymes and 2.78 and 2.37 mg sugar / ml / 24h of C_1 and Av enzymes, respectively) were obtained in medium was contained: 10 g cotton stalks, 10g $CaCO_3$ and 100 ml water. In dual step acid hydrolysis, the high yield of reducing sugar (80.0 %) was obtained with 85 min - first step acid hydrolysis and 60 min second step acid hydrolysis. While, high yield of reducing sugar in enzymatic hydrolysis (14.97 mg / ml) was occurred with solid cellulase.

Keywords : cotton stalks, *Aspergillus niger,* acid hydrolysis, enzymatic hydrolysis, low-cost media.

INTRODUCTION

Until now the saccharification of cellulose is still uneconomical .But with respect to cellulosic materials of more than 70 x 10^9 tons synthesized in nature each year, efforts have to be undertaken to make this inexhaustible resource directly or indirectly available , (Herr, et al 1978). Recently interest in the concentration of acid hydrolysis of biomass has increased, (Sen and Reddy, 1995). Most research efforts have been focused on pure cellulose whereas little attention has been paid to lignocellulose . But, experiments have shifted to lignocellulose, (Rivers and Emert 1988 and Mariamma and Kurup 1997) . Agricultural wastes facilitate more fungal growth than pure cellulose by inducing cellulase biosynthesis. Recent interest in the application of cellulase for the practical conversion of natural crystalline cellulose into simple sugar, (Lakshmikant 1990). The microbial conversion of cellulosic materials to useful compounds is necessary for the effective use of agricultural and forest resources, (Sukhumavasi *et al.* 1984). Fungi of the genus *Aspergillus* are important organism for a variety of biotechnological industries. Several species, are used for the production of secondary metabolites and various hydrolytic enzymes, (Schrickx *et al.* 1995). Cotton stalks is an agriculture waste, available in significant amount in Egypt and mainly used as firewood.

In the present study, cotton stalks, was subjected to various biochemical hydrolysis by cultivation of *A. niger* on cotton stalks in simple media; dual-step acid hydrolysis and enzymatic hydrolysis to evaluate the effects of these methods for hydrolysis of cotton stalks to reducing sugar and valuable products.

MATERIALS AND METHODS

Substrate: Milled cotton stalks (~ 0.5 cm in length, in a laboratory mill) was used in this study . Cotton stalks contained 49.8% cellulose ; 26.4% free - ash lignin ; 21.5 % hemicellulose and 2.3% ash (d.w) . The chemical composition of cotton stalks was determined by Kalininskaya and kultyshkina (1974) and Zadrazil and Brunnert (1980) methods for the evaluation of cellulose and free - ash lignin, respectively . The ash content was determined by burnet a sample of cotton stalks at ~400^{0}C to constant weight . While , hemicellulose content was calculated by the difference from 100%.

Microorganism : *Aspergillus niger* NRR-326 was obtained from the United States Dep. Agric. Cullure Collection , Peoria , Illinois.Stock Cultures of potato dextrose agar (PDA)slants were used for preservation of the microorganism at 4^{0}C and subcultured at two months intervales .

Inocula : Agar plugs of profus *A. niger* growth was inoculated into 100 ml . steril fermentation medium each contained in 250 ml flasks.

Biodegradation : Biodegradation of cotton stalks was occurred by cultivation of *A. niger* on cotton stalks in simple media as follows :-

1)Cotton stalks was hydrolyzed in Roch - Chui and Hang (1990) medium which contained 10g cotton stalks , 10g CaCO3 and 100 ml water were added to 250 ml. flask (medium I) .

2)Cultivation of *A. niger* on 4% cotton stalks as the sole carbon source and in the presence of 1% Tween 80 (medium II).

3)Corn-steep liquor (CSL) medium : CSL medium as recommended by Kadam and Newman (1997) contained 0.3% corn steep Liquor , 2.5 mM $MgSO_4$ - 7 H_2O and supplemented with 4% cotton stalks (medium III) . All the above mentioned media were sterilized in autoclave at 121^{0}C for 20 min , inoculated with agar plugs of profus *A. niger* and incubated in shaker (~120rpm) for six days at 30^{0}C . The bioproducts in cultures filtrate were evaluated as follows :-

(1) Reducing sugar was determind by a sub - microdetermination method of James and Marvin(1949) .

(2) Alph - amylase activity was determined according to Hayashida and Teramoto (1986) method .

(3) Amyloglucosidase activity was determined as the method described by Nagasaka et al (1998).

One unit (U) of enzyme activity was defined as the amount of enzyme releasing one micromol of reducing suger as glucose per ml . of culture filtrate per min under the assay conditions.

(4)Activities and composition of cellulolytic enzymes in cultures filtrate were evaluated according to the method of Galas , et at (1981).

Cx ; (endo - glucanase , saccharified , CMC) ; enzyme activity = mg sugar / ml enzyme / h .

C_1 ; (exo - glucanase, degrades Solka floc SW - 40) ; enzyme activity = mg sugar / ml enzyme / 24h .

A_v ; (B-glucosidase, saccharified Avicel SF) ; enzyme activity= mg sugar / ml enzyme / 24 h

X ; (xylanase , hydrolyzed xylan) ; enzyme activity = mg sugar / ml enzyme / h.

Dual - step acid hydrolysis : For the quantitative saccharification of cotton stalks , the recommended method by Saeman, et al (1945) was used. Reducing sugar in acid hydrolyzate was estimated as mentioned above .

Enzymatic hydrolysis : Milled cotton stalks at 0.5 g (d.w) were incubated in 10ml sodium acetate buffer (50 m.mol , pH 4.6) containing 0.1g solid cellulase or 0.1 ml celluclast enzyme in a 100 ml flask . The flask was held in water bath with shaking (~50 rpm) for three days at 37 ^{0}C . Reducing sugar in enzymatic hydrolysis was estimated .

Sources of cellulases : Solid Cellulase type II from *A. niger* was purchased from Sigma Co. liquid cellulolytic enzyme known by the trade name of celluclast was obtained from Novo Co. in Denmark.
One ml or gram of cellulolytic enzymes contained 327.5 and 289.9 units of Cx enzyme, 432.2 and 127.5 units of Av enzyme , 378 and 161.1 units of C1 enzyme and 566.5 and 660.4 units of X enzyme for celluclast and solid cellulase type II, respectively.

RESULTS AND DISCUSSION

(1) Bioproducts in cultures filtrate :-

Table (1) summerized the bioprducts in culture filtrate of cultivation of *A. niger* on the cotton stalks in simple media , after 6days with shaking (~120 rpm) at 30^{0}C. Data in Table (1) indicated that the three media were contained the same level of amyloglucosidase activity (1.27,1.33 and 1.17 U/ml/min,respectively).The levels of bioproducts in medium II was relatively at the same levels of bioproducts in medium III . Medium II and III were contained 1.01 and 0.98 mg/ml reducing sugar ; 1.34 and 1.30 mg sugar/ml/24h B-glucosidase (Av) and 5.22 and 5.16 mg sugar/ml/h endo-glucanase (C_x) , respectively .

Table (1): Bioproducts in cultures filtrate of different media of cotton stalks by cultivation of *A. niger* for six days with shaking (~ 120 rpm) at 30^{0}C.

Products in cultures filtrate	Media		
	I*	II	III
Reducing sugar mg/ml	1.72	1.01	0.98
∝-amylase U/ml	5.01	3.01	3.67
Amyloglucosidase U/ml	1.27	1.33	1.17
C_x mg sugar/ml/h	5.92	5.22	5.16
C_1 mg sugar/ml/24h	2.78	1.26	1.00
A_v mg sugar/ml/24h	2.37	1.34	1.30
X mg sugar /ml/h	9.31	7.42	8.50

*** after seven days of incubation**

Also, they were contained rather the same level of ∝ -amylase (3.01 and 3.67 U/ml/min , respectively) . But, medium I was contained the higher level of ∝ -amylase (5.01 U/ml/min). The maximum of xylanase activity was in medium I (9.31 mg sugar/ml/h) followed by medium III and II (8.50 and 7.42 mg sugar/ml/h), respectively . Also, medium I was contained the highest level of C_1 and A_v enzymes (2.78 and 2.37 mg sugar/ml/24h, respectively). Medium I slightly enhanced reducing sugar content (1.72 mg/ml) and C_x enzyme (5.92 mg sugar/ml/h) than medium II and III . These results were in agreement with the results of Chowdhury , at al (1991), who found that cultivation of *A. micromonospora* sp in basal salt medium supplemented with different substrates at 1% (w/v) for 3 days at 37 ^{0}C, the cultures filtrate were contained 0.67, 1.0 and 0.11 mg reducing sugar / ml ; 23.7 , 33.8 and 5.4 umol sugar / ml / min of C_x enzyme , and 0.59, 0.94 and 0.21 umol sugar / ml / min of Av enzyme , for bagasse , rice straw and cotton , respectively .
From the above mentioned results, it was found that the different media of *A. niger* showed variation in their components which depends on the microorganism, the cutlure conditions and the composition of substrate which induce or inhibit the biosynthesis of selected components of enzymes . It is observed that medium I was the best culture. It contained the highest levels of reducing sugar, ∝-amylase, amyloglcosidase, C_1 ,A_v and X enzymes than the two other media, and it is valiable as a low-cost medium for economical production of enzymes

(2) Dual-step acid hydrolysis : For the quantitative saccharification of cotton stalks , a series of experiments were performed in which cotton stalks was subjected to varying length of time and temperature during the first step acid hydrolysis (40,85 and 120 min . at 30 ^{0}C with 72% (v/v) H_2SO_4) .and second step acid hydrolysis (15 , 30 , 60 min. at 121 ^{0}C with 4% (v/v) H_2SO_4) . To optimize the yield of reducing sugar , these condition were studied .

Table (2): Reducing sugar % of cotton stalks subjected to different periods of primary and secondary steps acid hydrolysis .

Time of primary hydrolysis at 30^{0}C min	Time of secondary hydrolysis at 121^{0}C min	Reducing sugar %
40	15	64.60
	30	66.20
	60	74.10
85	15	70.08
	30	77.21
	60	80.00
120	15	72.13
	30	75.09
	60	79.94

Data in Table (2) indicated generally that increasing the time of hydrolysis in first and second steps of acid hydrolysis increased the % of reducing sugar . At 40 , 85 and 120 min of primary hydrolysis , increasing the time of secondary hydrolysis were increased reducing sugar % to (64.6 , 66.2 and 74.1) ; (70.1 , 77.2 and 80.0) and (72.1,75.1 and 79.9) % at 15 , 30 and 60 min of secondary hydrolysis , respectively . With these relation Saeman , *et al* (1945) found that the high yield of reducing sugar occurred from the hydrolysis of Douglass fire wood was 68.9% at 60 min of primary hydrilysis and 60 min secondary hydrolysis. While , at 30 and 45 min of primary hydrolysis were produced the same levels of reducing sugar (68.6) % after 60 min of secondary hydrolysis .

From the results in Table (2) , it was found that the reducing sugar % were the same as that obtained using 85 min. primary hydrolysis (80% reducing sugar) and 120 min primary hydrolysis (79.94 % reducing sugar) , after 60 min. of secondary hydrolysis to both. But 85 min primary hydrolysis was better than 120 min. primary hydrolysis because it is less time consumed for obtaining the same level of reducing sugar %.

(3) Enzymatic hydrolysis : Data in Table (3) show the saccharification of cotton stalks using two differnet sources of cellulases . Solid cellulase gave the highest content of reducing sugar (14.97 mg/ml, 29.94 reducing sugar %) . While celluclast enzyne gave 9.52 mg sugar/ml and 19.04 reducing sugar % . These results are in line with Chowdhury , *et al* (1991) who found that sacchrification of rice straw and bagasse at 37◦C using cellulase from A. *micromonospore* sp. grown on 1% alkaline preteated bagass and xylan produced (7.1 and 8.0) and (12.0 and 16.5) mg sugar/ml for rice straw and bagass , respectively after 3 days of hydrolysis . While Singh, *et al* (1990) found that treated 1% wheat straw , bagass and corn cabs with celluase from *A. niger* produced 1.1 , 1.5 and 1.2 mg sugar/ml with 10,14 and 11% saccarification after 2 days at 50 ^{0}C . Enzymatic hydrolysis of native lignocellulosics is generally a slow process. The lower succharification with lignocellulose may be due to the natural cellulosic substrate possess a high amount of lignin .

Table (3): Enzymatic hydrolysis of cotton stalks with different sources of cellulase (0.1 g or 0.1 ml enzyme) at 37 ^{0}C and pH 4.6 .

Kind of cellulase	Reducing sugar mg/ml	reducing sugar %
solid cellulase	14.97	29.94
celluclast	9.52	19.04

REFERENCES

Chowdhury , N. A. ; M. Moniruzzaman ; N. Nahar and N. Choudhury (1991). Production of cellulases and saccharification of lignocellulosics by *A. micromonospora* sp. World J. Microbiol . Biotechnol . , 7: 603-606.

Galas , E. ; R. Pyc ; K. Pyc And K. Siwinska (1981). Universal method for expressing activity of cellulolytic enzymes . Europ. J. Appl. Microbiol . Biotechnol ., 11 : 229-233

Hayashida, S. and Y. Teramoto (1986).: Production and charcteristics of raw starch digesting ∝-amylase from a protease negative *A. ficum* mutant . Appl . Environ . Microbiol. , 52, (5) : 1068 - 1073 .

Herr, D. ; G. Luck and H. Dellweg (1978). Formation of cellulases and degradation of cellulose by several fungi. J. Ferment Technol. , 56(4): 273-278.

James T, P. and J. Marvin (1949). A submicrodetermination of glucose . J. Biol . Chem ., 143 : 149-151 .

Kadam , K. L. and M. M. Newman (1997). Development of a low -cost fermentation medium for ethanol production from biomass . Appl. Microbiol . Biotechnol. 47 : 625- 629 .

Kalininskaya , T. A. and I. T. Kultyshkina (1974). Use of the anthrone method for determination of cellulose consumption in soil . Prikladnaya Biokhimiya i mikrobiologiya , 10, (4) : 618-620 .

Lakshmikant , K. (1990). Cellulose degradation and cellulase activity of five cellulolytic fungi . World . J. Microbiol , Biotechnol ., 6 : 64-66.

Mariamma , A. and G. M. Kurup (1997). Pretreatment studies of cellulose wastes for optimization of cellulase enzyme activity Appl . Biochem . Biotechnol ., 62 : 201-211.

Nagasaka, Y. ; K. Kurosawa ; A. Yokoto and F. Tomita (1998) : Purification and properties of the raw starch digesting glucoamylase from *Corticium rolfsii* . Appl. Microbiol . Biotechnol , 50 : 323-330.

Rivers , D. and G. Emert (1988) : Factors affecting the enzymatic hydrolysis of municipel - solid - waste components. Biotechnol . Bioeng ., 31 : 278-281

Roch- Chui, Y and Y. D. Hang (1990) : Amylolytic enzyme production by *Rhizopus oryza* grown on agricultural commodities . World J. Microbiol. Biotechnol ., 6 : 15-160.

Saeman, J. F.; J.L. Bubl and E. E. Harris (1945). Quantitative sacccharification of wood and cellulose. Ind Eng. Chem., 17,(1): 35-37.

Schrickx , J. M, ; A. H. Stouthamer and H. W. van Verseveld (1995). Growth behavior and glucoamylase production by *A. niger* N 402 and a glucoamylase overproducing transformant in recycling culture without a nitrogen source. Appl. Mirobiol . Biotechnol. , 43 : 109 -116.

Sen , k. K. and S. S. Reddy (1995). Acid hydrolysis of jute stick . Cellulose Chem . Technol., 29 : 151-158.

Singh, A. ; A. B. Abidi ; N. S. Darmwal and A.K. Agrawal (1990). Saccharification of cellulosic substrates by *Aspergillus niger* cellulase. World J. Microbiol. Biotechnol., 6 : 333-336.

Sukhumavasi, J. ; K. Ohmiya ; M. Suwana-Adth and S. Shimizu (1984). Conversion of tough cellulose to useful compounds by an anaerobe isolated from compost . J. Ferment . Technol. , 62 (6): 545-550 .

Zadrazil , F. and H. Brunnert (1980) : The influence of ammonium nitrate supplementation on degradation and in vitro digestibility of straw colonized by higher fungi . Europ. J. Appl . Microbiol. Biotechnol . , 9 : 37-44.

J. Agric. Sci. Mansoura Univ., 25 (7), July, 2000.

الاستفادة من حطب القطن

سكينه انور محمد سليمان
معهد بحوث الاراضى والمياه والبيئة - مركز البحوث الزراعية - جيزة - مصر

استخدمت ثلاث طرق مختلفة للاستفادة من حطب القطن لتحويله إلى سكر مختزل ومواد لها أهمية اقتصادية وتشمل هذه الطرق :

(١) المعاملة البيولوجية : عن طريق تنمية فطر الأسبرجلس نيجر على حطب القطن فى بيئات اقتصادية بسيطة التركيب (ثلاث بيئات) لمدة ٦ أيام مع الرج (~ ١٢٠لفة/دقيقة) على ٣٠ °م- وقدرت نواتج نشاط ونمو الكائن الحي فى راشح المزارع .

(٢) المعاملة بالحامض : واستخدم فى ذلك نظام التحليل ثنائي الخطوة - Dual-step acid hydrolysis وذلك على درجات حرارة وفترات تحليل مختلفة ففي المرحلة الأولى من التحليل استخدم ٧٢ % يد٢ كب ا٤ (ح/ح) على ٣٠°م لمدة ٤٠،٨٥،١٢٠ دقيقة - أما المرحلة الثانية استخدم فيها ٤% يد٢ كب ا٤ (ح/ح) على ١٢١° م لمدة ١٥،٣٠، ٦٠دقيقة.

(٣) المعاملة الأنزيمية: استخدم فيها مصدرين من انزيمات السليوليز (كل على حدة) وذلك على°٣٧م ، رقم حموضة ٤,٦ لمدة ٣ أيام. **وكانت أهم النتائج:**

(أ) فى المعاملة البيولوجية كان آعلى نواتج نشاط ونمو الفطر: ١,٧٢ ملليجرام سكر مختزل/ملليلتر ؛ ٥,٠١، ١,٢٧ وحدة انزيم/ملليلتر الفا - اميليز ، اميلو جلوكوسيديز ؛ ٥,٩٢ ، ٩,٣١ ملليجرام سكر/ملليلتر/ساعة من C_x ، x ، انزيم ؛ ٢,٧٨ ، ٢,٣٧ ملليجرام سكر/ملليلتر/٢٤ساعة من C_1 ، A_v انزيم على الترتيب وذلك فى البيئة المكونة من ١٠ جرام حطب قطن + ١٠ جرام كربونات كالسيوم +١٠٠ ملليلتر ماء .

(ب) المعاملة بالحامض فكان آعلى إنتاج ٨٠% سكر مختزل على ٨٥ دقيقة من مرحلة التحليل الأولى على ٣٠ ^{0} م ، ٦٠ دقيقة من مرحلة التحليل الثانية على°١٢١م.

(جـ) المعاملة الأنزيمية فكان آعلى محتوى ١٤,٩٧ ملليجرام جلوكوز/ملليلتر يقابله ٢٩,٩٤ % سكر مختزل وذلك باستخدام الأنزيم الصلب .