ESACCHARIFICATION OF SOME LIGNOCELLULOSIC MATERIALS BY *TRICHODERMA* AND *ASPERGILLUS* SPECIES AND CELLULASES ACTIVITY

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Mohamoud, Sara, M.[(1)]; Abou-Taleb, Khadiga, A.[(2)]; Shaarawy, H. H.[(3)]; Ramadan, E. M.[(2)] and ElKassas, H. I.[(1)]

1) Department of Agricultural Sciences, Institute of Environmental Studies and Research, Ain shams University 2) Department of Agricultural Microbiology, Faculty of Agriculture, Ain shams University 3) Chemical Engineering Department, Research Division, NRC.

ABSTRACT

Biodegradation of Lignocellulosic wastes (cotton stalks and corn cobs) was carried out by single and mixed cultures of *Trichoderma viride* T3 and three strains of *Aspergillus niger* with code A2, A4 and An2. Results indicated that biodegradation of cotton stalks and corn cobs by mixed culture gave higher activities for cellulase production and saccarification than single cultures. Mixed culture of *Trichoderma viride* T3 and *Aspergillus niger* A4 gave the highest activity of cellulase being 1.02 & 0.94 U/ml with 49.7 & 45.8 % of saccarification on media containing alkaline corn cobs and cotton stalks, respectively. It was observed that the ability of the mixed for degrading corn cobs was more efficient than degrading cotton stalks which increased about 1.08 fold of cellulase activity and saccharification. The biological and enzyme parameters of co-culture on alkaline corn cobs were calculated, which recorded 56.73 % of yield factor, 85.59 % of sugar utilization efficiency, 16.60 % of effective yield, 32.55 % enzyme yield, 37.60 % conversion coefficient with productivity of 0.15 U/ml/d.

Keywords: Cellulase activity, Lignocellulosic materials, saccharification, *Aspergillus niger*, *Trichoderma viride*.

INTRODUCTION

In Egypt, crop residues are byproducts of common crops such as cotton, wheat, maize and rice, with total amount of about 16 million tons of dry matter per year. Cotton crop area accounts for about 5% of the cultivated area in Egypt (El Saeidy, 2004).

Cotton stalks produced annually as agricultural residues reached 1.9 million tons (Mona, *et al.*, 2001). These post-harvest byproduct cause many severe problems, fires causing significant environmental and health disorders (Fouad *et al.,* 2010).

As a renewable raw material, corncobs is considered a potential feedstock for the production of biogas, biodiesel and bioethanol to fulfil the increasing demand for biofuels (Ioannidou *et al*., 2009). The hydrolysates of corncobs are therefore perfectly suited for biodiesel production using yeasts. These species of oil-producing yeasts accumulate up to 50% of fat in their dry mass (Kitcha and Cheirsilp, 2011).

Corncobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. These polymeric fibers consist of monomeric molecules. Cellulose is built of C6 sugars; hemicellulose mainly of the C5 sugars (xylose and arabinose). Lignin consists of phenolic macromolecules (Pointner *et al*., 2014).

Pretreatment aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break lignin seal. Pretreatment makes cellulose more accessible to enzymes so that conversion of

carbohydrate polymers into fermentable sugars can be achieved more rapidly and with more yields (McMillan *et al*., 1994).

The major methods include pretreatment by milling (Delgenés *et al*., 2002), acid hydrolysis (Taherzadeh and Karimi, 2007), steam explosion (Mukhopadhyay and Fangueiro, 2009), hot water (Liu and Wyman, 2005) and alkaline hydrolysis (Goswami *et al*., 2009 and Binod *et al*., 2010). Usually grinding and milling are the initial steps of pretreatment of any biomass which reduces the particle size, though the combination of grinding with other pretreatment method. Superfine grinding of steam exploded biomass has been proved better than ground residue when hydrolyzed though energy required for the process also has to be considered while going for commercial applications (Zhu *et al*., 2006).

There is a large number of fungi play a vital role to degrade these wastes contain cellulose into sugar such as *Trichoderma aureoviride*, *T.ressi*, *T.koningii* BTS120 and *Aspergillus* sp. (Bahaa *et al.*, 2011; Fang and Xia, 2013 and Rana *et al.*, 2014).

The aim of the work was to investigate the saccharification of lignocellulosic materials (cotton stalks and corn cobs) into sugar by fungal cultures. The biological parameters of the tested fungi were also elucidated.

MATERIAL AND METHODS

Fungi used: Three isolates of *Aspergillus* sp. and one isolate of *Trichoderma* sp. used in this study, as cellulytic fungi, were obtained from Microbiology Department, Fac. of Agric., Ain Shams University, Cairo, Egypt.

Media used:

Medium (1), Potato dextrose agar (PDA), was used for maintenance and preservation of the tested fungi, described by **(Difco Manual, 1984).** The same medium was used without agar as broth medium.

Medium (2), Basal medium **(Mandels *et al.*, 1969).** It was used to study cellulase activity and saccharification determination. Its composition was as follows (g/L): Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2; $CaCl_2$, 0.3; $MgSO_4$, 0.3; Yeast extract, 0.25; Peptone, 0.75; Trace elements (mg/L): $FeSO_4.7H_2O$, 5; $COCl_2$, 20; $MnSO_4$, 1.6 and $ZnSO_4$, 1.4 then adjusted pH to 7.0.

These media were autoclaved at 121^oC for 15 min.

Buffers and solutions

- **Citrate buffer (0.05M)** consists of : Solution (a) 0.05 M citric acid (10.51 g/L) and solution (b) 0.05 M trisodium citrate (14.71 g/L), adjusted pH to 4.8 by adding 667 ml solution (a) to 1 liter of solution (b) (Mandels *et al.*, 1969).
- **Carboxymethlycellulase (CMC) solution 1% (Mandels *et al.*, 1969).** Its composition was as follows: CMC 10 g/L and adjusted pH to 4.8.

Lignocellulosic agricultural wastes: Agricultural wastes (cotton stalks and corn cobs) were collected from Kafr El-Dawar, El-Bahera Governorate.

Cotton stalks and corn cobs were used as samples of agriculture wastes, which harvested from field, and solid biomass was washed with tap water until clean, then dried at 80°C overnight (Yonghao *et al*., 2016). The dried biomass was milled, as mechanical pretreatment (MT1), then using different pretreatments, being thermal pretreatment (TT2) by heating at 121^oC/1h and

physiochemical pretreatment by acid (AT3) was carried out using H_2SO_4 10% /1h (Noriko *et al*., 2005) or alkaline (KT4) was done with NaOH at concentrations of 10 % for 1h (Singh *et al*., 2011).

The pretreated biomass were filtered with two layers of muslin cloth, and washed with distilled water. Then, biomass was dried at 50±2°C and subsequently used for enzymatic hydrolysis experiments.

Maintenance of cultures: Stocks culture slants were grown on PDA medium at 30^{o}C for 3-5 days and maintained at 5^{o}C.

Standard inoculants: The tested fungal isolates grown on PDA slants for 3 days at 30^{o}C and were used to prepare the spore suspensions by adding 10 ml of sterile saline solution (0.95 % NaCl) water to each fungal agar slant and gently scraping with sterile inoculation loop. The obtained spore suspensions (1×10^{10}/ml) were used as fungal standard inoculants for flasks experiments.

Submerged fermentation process for saccharification (cellulase production): It was carried out in 250 ml plugged Erlenmeyer flasks containing 100 ml of basal medium supplemented with 1% (w/v) pretreated agricultural waste samples and then inoculated with 5% (v/v) of standard inoculants of the tested fungal isolates. The inoculated flasks were incubated at 30^{o}C on a rotary shaker at 100 rpm / min for 7 days.

The fermented medium was filtrated through whatman No.1 filter paper to separate mycelial mat to determine the cell dry weight. The culture filtrate was used to determine enzyme activity, extracellular consumed sugar. Some biological parameters were calculated (Diener *et al*., 2004).

Identification of pioneer tested fungal isolates: The most efficient fungal isolates for saccharification were identified based on the morphological appearance under light microscope (shape and conidia) and cultural characteristics according to Barnett and Hunter (1998).

Analytical procedures

Fungal count: The number of fungal spores was counted in the filtrate using haemocytometer slide **(Kolmer *et al.*, 1959)**. Fungal dry weight was determined by separate the mycelium from broth culture using filter paper (Whatman No.1) and drying at 80^{o}C to constant weight.

Dry cell mass determination (Srilekha *et al.*, 2011):For dry cell mass determination, 10 ml of culture samples were filtered, washed and dried to a constant mass at 104^{o}C.

pH determination: pH of culture was measured using pH-meter model (Microprocessor 211) equipped with glass electrode.

Reducing sugar determination: Glucose was determined using glucose oxidase peroxidase kits (GOD-POD. Liquid) from EL NASER PHARMACEUTICAL CHEMICALS CO. (Egypt) using spectrophotometer (JENWAY 6300) and measured at 546 nm (Kaplan *et al*., 2001).

Enzyme assay: Carboxymethyl-cellulase (CMCase) activity was assayed using a method suggested by Mandels *et al.* (1962). The activity was estimated using 1 % solution of carboxymethlycellulase (CMC) in 0.05 M citrate buffer (pH 4.8) as a substrate. The reaction mixture contained 1 ml citrate buffer, 0.5 ml of substrate solution and 0.5 ml of suitably diluted enzyme solution. The reaction was carried out at 50°C for 30 min. The

amount of reducing sugar released in the hydrolysis was measured. One unit of CMCase activity was expressed as 1 μ mol of glucose liberated per ml enzyme per minute.

Parameters calculation:

Yield factor (Y) (Herbert *et al.*, 1971) = ((Growth (dry weight) / Consumed sugar)) × 100

Enzyme Yield (EY) (Ramadan *et al.*, 1985) = ((Enzyme activity (U/ml) / Original sugar (gl^{-1})) × 100

Effective Yield (YE) = ((Fungal biomass) / (Initial substrate concentration)) × 100

Substrate Utilization Efficiency (SUE) = ((Effective yield (YE) / Yield factor (Y)) × 100

Sugar utilization efficiency (Ramadan *et al.*, 1985) = (Consumed sugar / Initial sugar)

Productivity (P) (Lee *et al.*, 1996) = ((Enzyme activity (Uml^{-1}) / Fermentation time (h))

Conversion coefficient (CC) = ((Enzyme activity (Uml^{-1}) / Utilized sugar ($gl^{-1)}$)) × 100

Saccharification Conversion (SC) (Velayudhan *et al.*, 2104) = ((Enzyme activity mg/ml) × 0.9) / (Initial Substrate concentration)) × 100

Statistical experimental analysis: The collected data were statistically analyzed using **IBM® SPSS® Statistics software (2011)**. The correlation coefficient was analyzed using Microsoft Office Excel 2013.

RESULTS AND DISCUSSION

Collection and identification of fungal isolates:

In the present study, 4 fungal isolates were collected from Microbiology Dept., Fac. of Agric., Ain shams Univ. They were used as cellulase producers. These isolates belong to *Trichoderma* sp. with code T3 and *Aspergillus* sp. with codes A2, A4 and An2.

These fungal isolates were identified depending on their cultural and morphological characteristic. *Trichoderma* sp. T3 isolate was classified as *Trichoderma viride*, which giving colonies on PDA agar with broadly spreading, hyaline with fruiting areas appeared as tufts, white at first and becoming in deep green shades with colorless reverse. Conidiophores arised as branches of mycelium, dichotomously branched, occasionally forming whorls (Fig. 1d).

Whereas, all *Aspergillus* sp. isolates with codes A2, A4 and An2 were identified as *Aspergillus niger*, which giving colonies on PDA agar medium rapidly growing with abundant submerged mycelium. Reverse usually without color. Conidiophores mostly arise directly from substratum, conidial heads fuscous, blackish brown, small, most columnar masses of a few conidial chains (Fig. 1a-c).

Among the filamentous fungi of environmental importance are *Trichoderma* sp. and *Aspergillus* sp., their hydrolytic efficiency is as a result of secretion of extracellular enzymes such as cellulases, hemicellulases and ligninases. Of these, cellulase is the most important and a complex enzyme

that acts synergistically though often described as contrasting faces of a single enzymatic capability (Milala *et al*., 2014).

Pretreatment makes cellulose more accessible to enzymes so that conversion of carbohydrate polymers into fermentable sugars can be achieved more rapidly and with more yields (McMillan *et al.,* 1994).

Therefore, an experiment was carried out to investigate the effect of four different pretreatments of agricultural wastes (cotton stalks and corn cobs) on saccharification and cellulase production by single and mixed fungi (Tables 1-2). It was found that lignocellulosic wastes were more biodegradable by mixed fungal cultures (*Trichoderma viride* T3 & *Aspergillus niger* A2; *Trichoderma viride* T3 & *Aspergillus niger* A4 or *Trichoderma viride* T3 & *Aspergillus niger* An2) than single culture (*Trichoderma viride* T3, *Aspergillus niger* A2, *Aspergillus niger* A4 or *Aspergillus niger* An2), it could be due to produce a large and more efficient enzymes necessary to breakdown hemicelluloses and cellulose (Nigam *et al.*, 2009).

Results demonstrated that the co-culture of *Trichoderma viride* T3 & *Aspergillus niger* A2, *Trichoderma viride* T3 & *Aspergillus niger* A4 or *Trichoderma viride* T3 & *Aspergillus niger* An2 achieved the highest degradation significant effect of pretreatments cotton stalks and corn cobs, which gave the maximum yield of biomass ranged from 0.90 – 1.39 g/L and 1.00 – 1.66 g/L, sugar consumption between 2.38- 2.64 g/L and 2.72 – 2.97 g/L and cellulase activity ranged from 0.67 – 0.94 U/ml and 0.70 – 1.02 U/ml with % of saccharification ranged from %, 32.5 – 45.8 % and 34.1 – 49.7 %,

respectivly

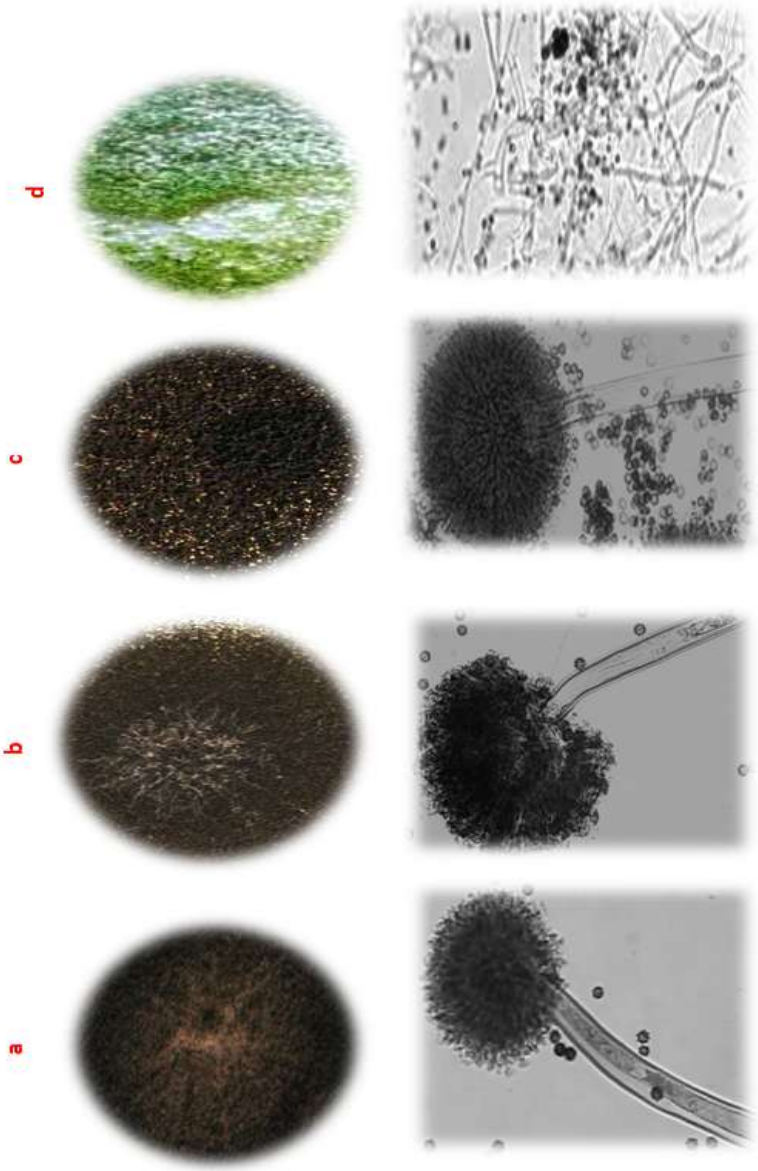

Fig. 1. Identification of fungal isolates based on phenotypic properties (the cultural and morphological shape).
a) *Aspergillus niger* A2 b) *Aspergillus niger* A4 c) *Aspergillus niger* An2 d) *Trichoderma viride* (T3)
Magnification 1000 X and 400 X, respectively.

Furthermore, results indicated that the highest biodegradation (saccharification, biomass and enzyme activity) were achieved by the tested fungi when propagated on wastes after pretreatment with physicochemical by 10 % NaOH (KT4 treatment) followed by 10 % H_2SO_4 (AT3 treatment), then physical treatment with heat at 121^{o}C (TT2 treatment) and milling (MT1 treatment). So, it was observed that physicochemical pretreatment of cotton stalks and corn cobs with 10 % NaOH was the best one for biomass production being ranged from 1.28 - 1.39 g/L and 1.37 - 1.66 g/L with saccharification ranged from 41.7 - 45.8 % and 41.8 - 49.7 % and enzyme activity ranged from 0.86 - 0.94 U/ml and 0.86 - 1.02 U/ml, respectively.

The co-culture of *T. viride* T3 and *A. niger* A4 gave the maximum saccharification (45.8 and 49.7 %), biomass (1.39 and 1.66 g/L) and enzyme activity (0.94 and 1.02 U/ml) on media supplemented with alkaline pretreatment of cotton stalks and corn cobs, respectively.

Moreover, it was observed that the saccharification and enzyme activity by mixed fungi *T. viride* T3 & *A. niger* A4 on alkaline wastes of cotton stalks and corn cobs were high significant at $p \leq 0.05$ and increased about $\simeq$ 1.2 folds as compared to single fungal culture of *T. viride* T3 or *A. niger* A4, respectively.

The biological activity of the tested fungi (single and mixed) were calculated and illustrated by Figs. (2-3). The highest figures of all calculated parameters of yield factor, effective yield, substrate utilization efficiency, sugar utilization efficiency, conversion coefficient, enzyme yield and productivity were recorded by co-culture of *T. viride* T3 & *A. niger* A4 on all

pretreatments lignocelluloses waste as compared with another the tested fungi.

The maximum parameters were obtained by *T. viride* T3 & *A. niger* A4 on alkaline pretreatment (KT4 treatment) of cotton stalks and corn cobs being 55.19 and 56.73 % of yield factor, 13.85 and 16.60 % of effective yield and 84.08, 85.59 % of sugar utilization efficiency and 26.4, 29.7 % of substrate utilization efficiency, respectively. Moreover, the highest productivity and enzyme yield were 0.14 and 0.15 U/ml/d, 30.06 and 32.55 % with conversion coefficient being 34.93 and 37.60 % on cotton stalks and corn cobs, respectively.

The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass (Tarkov and Feist, 1969).

Results in Table (3), confirmed that mixed cultures of *T. viride* T3 & *A. niger* A4 breakdown alkaline corn cobs with high efficiency than alkaline cotton stalks which gave a high values of biomass (1.66 g/L), enzyme activity (1.02 U/ml) and % saccharification (49.7%). From statistically analysis, it was observed a high positive correlation coefficient (r) between biomass and each of saccharification and cellulase production by selected mixed culture of *T. viride* T3 & *A. niger* A4 on alkaline pretreatment of cotton stalks and corn cobs ranged from 0.92 – 0.93 and 0.88 – 0.89, respectively.

Table (1): Physicochemical pretreatments of cotton stalks for biomass, consumed sugar and cellulase production by single and mixed fungi using flasks as a batch culture at 30^{o}C for 7 days.

Treatments		Tested fungi							
		Single				Mixture			
	Parameters	T3	A4	A2	An2	T3&A4	T3&A2	T3&An2	Mean
	C.D.W(g/L)	0.61	0.69	0.65	0.66	1.15	1.1	1.0	0.83^{d}
	Saccharification (%)	28.42	34.1	30.4	30.1	35.7	34.0	32.5	32.2^{c}
MT1	Consumed sugar(g/L)	2.22	2.26	2.27	2.25	2.54	2.43	2.45	2.34^{b}
	CMC activity(U/ml)	0.58	0.7	0.63	0.62	0.74	0.7	0.67	0.66^{c}
	C.D.W(g/l^{-1})	0.88	0.89	0.75	0.81	1.21	1.00	0.90	0.91^{c}
	Saccharification (%)	38.43	40.78	40.24	39.4	42.7	41.5	42.0	34.7^{b}
TT2	Consumed sugar(g/L)	2.48	2.49	2.5	2.51	2.64	2.53	2.53	2.36^{b}
	CMC activity(U/ml)	0.79	0.84	0.83	0.81	0.88	0.85	0.86	0.71^{b}
	C.D.W(g/l^{-1})	0.96	0.98	0.84	0.84	1.25	1.06	1.03	0.99^{b}
	Saccharification (%)	30.73	35.0	33.4	33.9	36.9	36.6	36.7	40.3^{a}
AT3	Consumed sugar(g/L)	2.42	2.31	2.24	2.11	2.61	2.46	2.38	2.38^{b}
	CMC activity(U/ml)	0.63	0.72	0.69	0.7	0.76	0.75	0.76	0.83^{a}
	C.D.W(g/l^{-1})	1.0	1.16	1.0	1.1	1.39	1.31	1.28	1.17^{a}
	Saccharification (%)	36.9	39.2	38.0	37.8	45.8	42.4	41.7	40.7^{a}
KT4	Consumed sugar(g/L)	2.45	2.41	2.27	2.16	2.51	2.46	2.43	2.52^{a}
	CMC activity(U/ml)	0.76	0.81	0.78	0.78	0.94	0.87	0.86	0.84^{a}
Mean	C.D.W(g/L)	0.92^{d}	0.86^{e}	0.84^{e}	0.8^{e}	1.2^{a}	1.1^{b}	1.0^{c}	
	Saccharification (%)	37.2^{c}	35.5^{d}	35.2^{d}	33^{e}	40^{a}	38.6^{b}	38.^{b}	
	Con. Sugar (g/L)	0.76^{c}	0.73^{d}	0.72^{d}	0.7^{e}	0.8^{a}	0.8^{b}	0.8^{b}	
	CMC activity (U/ml)	2.4c^{d}	2.3^{d}	2.32^{e}	2.2^{f}	2.5^{a}	2.4^{b}	2^{b,c}	

*****Initial total sugar of pretreated cotton stalks = 3.14 (g/L), CMC = carboxy methyl cellulose, C.D.W. = cell dry weight, Con. sugar = consumed sugar, MT1 = mechanical treatment, TT2 = thermal treatment, AT3 = acid treatment, KT4 = alkaline treatment, T3 = *T. viride*, A4 = *A. niger* A4, A2 = *A. niger* A2, An2 = *A. niger* An2. Values are means of 3 replica, Values in the same column followed by same letter do not significantly different from each other, according to Duncan's (1955) at 5 % level.

Table (2): Physicochemical pretreatments of corn cobs for biomass, consumed sugar and cellulase production by single and mixed fungi using flasks as a batch culture at 30^{o}C for 7 days.

Treatments		Tested fungi							
		Single				Mixture			
	Parameters	T3	A4	A2	An2	T3&A4	T3&A2	T3&An2	Mean
	C.D.W(g/L)	0.62	0.67	0.63	0.62	1.2	1.0	1.0	0.81^{c}
	Saccharification (%)	28.48	34.15	30.5	30.1	36.2	34.6	34.1	31.23^{c}
MT1	Consumed sugar(g/L)	2.56	2.58	2.61	2.58	2.88	2.76	2.79	2.67^{c}
	CMC activity(U/ml)	0.59	0.74	0.68	0.65	0.74	0.73	0.70	0.66^{d}
	C.D.W(g/L)	0.74	0.77	0.67	0.67	1.33	1.18	1.1	0.91^{b,c}
	Saccharification (%)	39.33	41.3	40.8	39.2	43.6	42.4	39.9	34.64^{b}
TT2	Consumed sugar(g/L)	2.83	2.85	2.87	2.86	2.97	2.92	2.94	2.73^{b,c}
	CMC activity(U/ml)	0.81	0.85	0.84	0.81	0.9	0.87	0.87	0.73^{c}
	C.D.W(g/L)	0.78	0.83	0.78	0.78	1.45	1.35	1.39	1.05^{a,b}
	Saccharification (%)	33.3	35..9	35.1	34.3	38.3	36.9	36.0	39.1^{a,b}
AT3	Consumed sugar(g/L)	2.82	2.75	2.65	2.53	2.82	2.81	2.72	2.78^{b}
	CMC activity(U/ml)	0.68	0.74	0.72	0.71	0.79	0.76	0.77	0.84^{a,b}
	C.D.W(g/L)	0.93	0.94	0.89	0.88	1.66	1.4	1.37	1.15^{a}
	Saccharification (%)	42.12	42.3	39.3	40.58	49.7	42.1	41.8	42.8^{a}
KT4	Consumed sugar(g/L)	2.85	2.8	2.77	2.59	2.93	2.81	2.79	2.89^{a}
	CMC activity(U/ml)	0.87	0.87	0.81	0.83	1.02	0.86	0.88	0.88^{a}
Mean	C.D.W(g/L)	0.8^{c}	0.76^{c}	0.7^{c}	0.72^{c}	1.4^{a}	1.2^{b}	1.1^{a,b}	
	Saccharification (%)	36.4^{b}	36 ^{b}	35.6^{b}	30.5^{c}	41^{a}	39^{a}	38.4^{a}	
	Con. Sugar (g/L)	2.7^{b,c}	2.7^{b,c}	2.6^{b,c}	2.58^{c}	2.9^{a}	2.8^{b}	2.8^{a,b}	
	CMC activity (U/ml)	0.68^{c}	0.67^{c}	0.67^{c}	0.67^{c}	0.8^{a}	0.7^{b}	0.67^{b}	

***** Initial total sugar of pretreated corn cobs = 3.47 (g/L), CMC = carboxy methyl cellulose, C.D.W. = cell dry weight, Con. sugar = consumed sugar, MT1 = mechanical treatment, TT2 = thermal treatment, AT3 = acid treatment, KT4 = alkaline treatment, T3 = *T. viride*, A4 = *A. niger* A4, A2 = *A. niger* A2, An2 = *A. niger* An2. Values are means of 3 replica, Values in the same column followed by same letter do not significantly different from each other, according to Duncan's (1955) at 5 % level

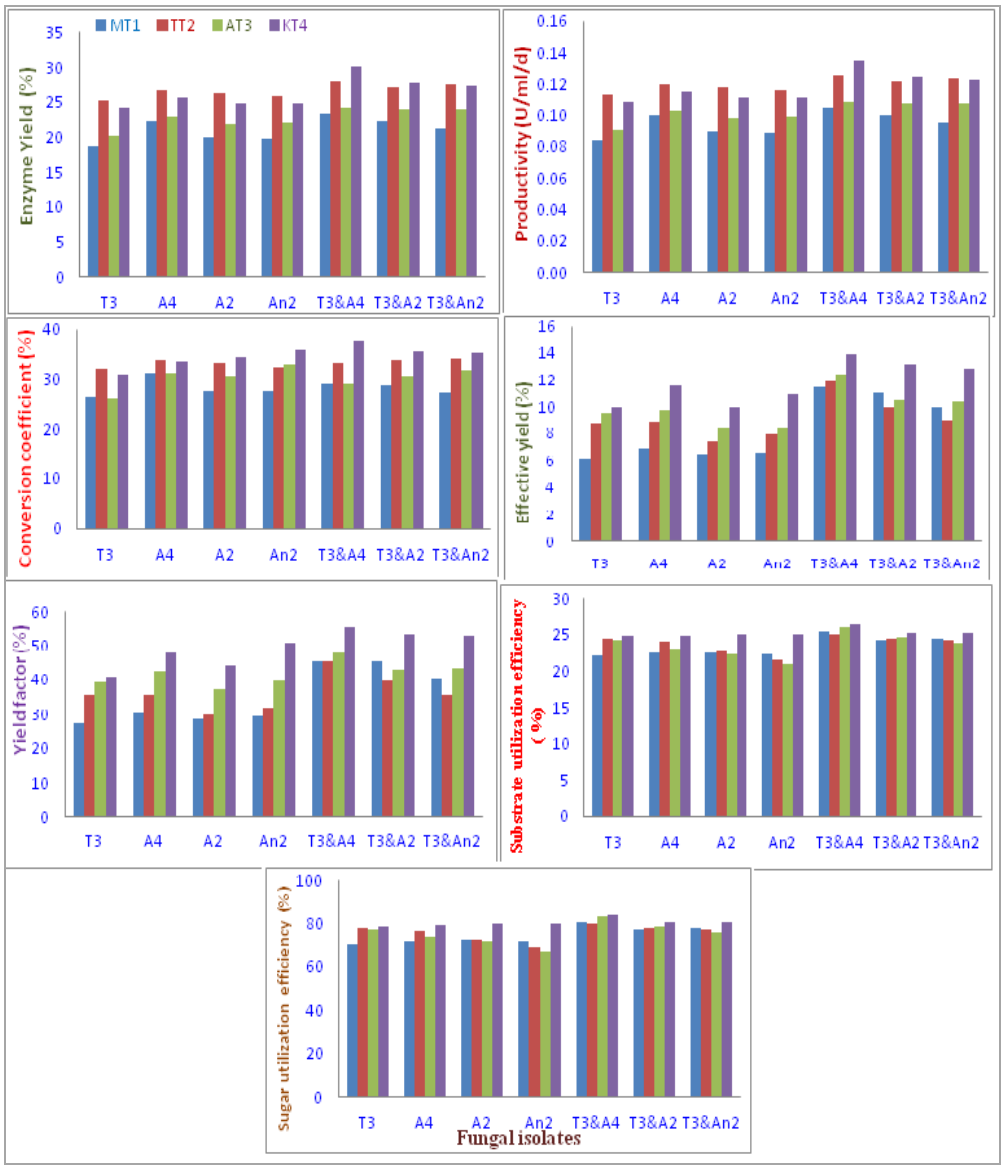

Fig. (2): Biological and enzyme activity parameters of single and mixed tested fungi on media containing of cotton stalks after mechanical (MT1), thermal (TT2), acid (AT3) and alkaline (KT4) treatments using flasks as batch culture for 7 days at 30°C.

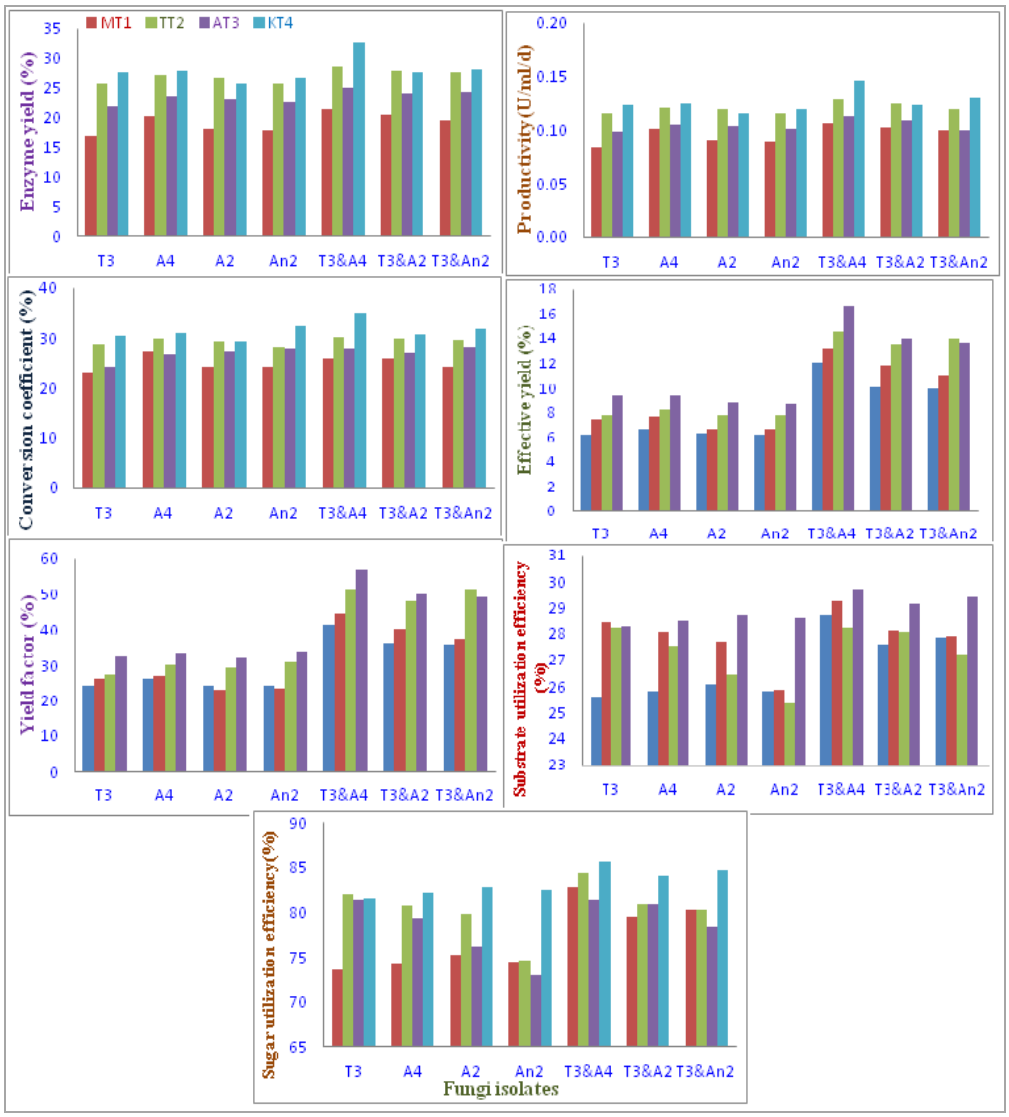

Fig. (3): Biological and enzyme activity parameters of single and mixed tested fungi on media containing of corn cobs after mechanical (MT1), thermal (TT2), acid (AT3) and alkaline (KT4) treatments using flasks as batch culture for 7 days at 30°C

Table (3): Comparative data for biomass, saccharification and cellulase production by *T. viride* T3 & *A. niger* A4 as influence by alkaline pretreatment of lignocellulosic wastes

Parameters	Alkaline hydrolyzed agro-industrial wastes				
	Saccharification %	Biomass (g/L)	Cellulase activity (U/ml)	**rBc**	**rBs**
Cotton stalks	45.87[b]	1.39[b]	0.94[b]	0.89	0.93
Corn cobs	49.68[a]	1.66[a]	1.02[a]	0.88	0.92

* rBs = Correlation coefficient between biomass and sachharification.
rBc = Correlation coefficient between biomass and cellulase.
Values in the same column followed by the same letter do not significantly different from each other, according to Duncan's (1955) at 5% level.

Therefore, alkali pretreatment has become one of the most promising methods used to degrade lignin in biomass, decrease the polymerization and crystallini structure of cellulose and thus to ease the enzymatic hydrolysis process (Jeya *et al*., 2009 and Parameswaran *et al*., 2010). Alkaline pretreatment is the most effective methods compare with acid (Shuhaida and Soh, 2016). The alkali pretreatment can result in a sharp increase in saccharification yields, pretreatment using NaOH is one of the effective pre-treatments, and could digest the hardwood from 14% to 55% by reducing the lignin composition from 55% to 20% (Balat, 2011 and Behera *et al.*, 2014). Also, Hashem *et al*. (2013) was proved that lignocellulosic biomass could not be enzymatically saccharified to high yields without a pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzymatic attack.

In addition, Nathalie *et al*. (2003) discussed the main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by

breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass. Iroba *et al.* (2013) and Cabrera *et al*. (2014) also stated that physicochemical pretreated with NaOH is the most effective method to break down the lignin.

However, Hashem *et al*. (2013) reported that non-crystalline and microcrystal cellulose dissolved in 8–10 % NaOH aqueous solution, indicating the increase of the electron cloud density, as a result of the interactions between cellulose chains with NaOH, which led to the rupture of the intermolecular hydrogen bonds. On that basis, cellulose filaments with small swelling ratio and low fibrillation nature in water were spun (Sen *et al*., 2016).

From the previous results, it could be conducted that pretreatment of lignocelluloses with NaOH was the best one. Also, corn cobs was better agricultural waste than cotton stalks for saccarification and cellulase production by mixed fungal cultures of *T. viride* T3 & *A. niger* A4 as compared to another wastes and another fungi.

So, it could be stated that alkaline pretreatment and mixed culture *T. viride* T3 & *A. niger* A4 were selected for further studies.

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تسكر بعض المواد الليجنوسلليلوزية بواسطة أنواع من TRICHODERMA و ASPERGILLUS ونشاط السيلوليز

[٥]

ساره محمد محمود[١]– خديجه أحمد أبو طالب[٢]– حسن حسين شعراوي[٣]
الشحات محمد رمضان[٢]– هشام ابراهيم القصاص[١]

١) قسم العلوم الزراعية والبيئية، معهد الدراسات والبحوث البيئية، جامعة عين شمس ٢) قسم الميكروبيولوجيا الزراعية، كليه الزراعة، جامعة عين شمس ٣) قسم الهندسة الكيميائية، المركز القومي للبحوث

المستخلص

تم التحلل البيولوجي لبعض المواد الليجنوسلليلوزية (حطب القطن و قوالح الذرة) بواسطه *Trichoderma viride* (T3) وثلاثة عزلات اخري من *Aspergillus niger* (A2، AN2، A4) ، سواء استخدام الفطريات منفردة او مختلطة.

وأظهرت النتائج أن التحلل البيولوجي لحطب القطن وقوالح الذرة من خلال الفطريات المختلطة كان افضل كفاءة لإنتاج السيلوليز والتسكر من الفطريات المنفردة. وأعطت الفطريات المختلطة من *T. viride* T3 & *A. niger* A4 أعلى نشاط للسيلوليز بنسبة ١,٠٢ – ٠,٩٤ وحده/مل مع ٤٩,٧ – ٤٥,٨ ٪ من التسكر على بيئة تحتوي على قوالح الذرة وحطب القطن المعامل قلويا، على التوالي. ولوحظ أن قدرة الفطريات المختلطة لقوالح الذرة كانت أكثر كفاءة من حطب القطن والتي زادت بمقدار حوالي ١,٠٨ أضعاف نشاط السيلوليز والتسكر. تم حساب معاملات البيولوجية وكفاءة الانزيم بواسطة الفطريات المختلطة *T. viride* T3 & *A. niger* A4 على قوالح الذرة القلوية والتي سجلت ٥٦,٧٣ ٪ معامل الانتاج، ٨٥,٥٩ ٪ كفاءة استهلاك السكر، ١٦,٦٠ ٪ من كفاءه الانتاج، ٣٢,٥٥ ٪ كفاءه انتاج الانزيم، معامل التحويل ٣٧,٦٠ ٪ مع انتاجية الانزيم ٠,١٥ وحده/مل/يوم.

الكلمات الدالة: نشاط السيلوليز، المواد الليجنوسليللوزية، التسكر، *Trichoderma viride*، *Aspergillus niger*.