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Research Article

Effect of EDTA on Production of Xylanase by Streptomyces Species and their Bleaching Effect on Papyrus and Cotton Stalk Pulp

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Xylanase production using Sterptomyces rochei and Streptomyces chromofuscus was improved with untreated and treated different pulps as substrate. Xylanase was produced on treated pulp with a maximum activity of 43.01 u/ml. Streptomyces chromofuscus exhibited a higher xylanase activity using either untreated or treated pulp as substrate rather than Sterptomyces rochei it was found that kappa number decreased from 54 in case of control to 32.8. Streptomyces rochei but 30.2 in case of Streptomyces chromofuscus Also brightness was enhanced from14.1% in case of control to 10.4% and9.5% in case of treated with Streptomyces rochei and Streptomyces chromofuscus respectively. Lignin % was enhanced by treated by Streptomyces rochei from 27% to16.4% and to 15.1% in case of Streptomyces chromofuscus EDTA had great influence of activate enzyme xylanase to achieve excellent bleaching IR,X-ray and electron microscope were also studied.

Key words: EDTA, bleaching, Streptomyces Species, xylanase activity, Cotton Stalk Pulp, Papyrus

INTRODUCTION

Xylan is widely distributed in plant cell walls and forms a main part of the hemicelluloses fraction (Wikerham and Kurtzman,1975). In some higher plants and agricultural wastes, xylan constitutes from 20-40% of the dry weight (Detroy, 1981 and Petterson, 1984). Xylan together with hemicelluloses forms the second most abundant renewable polysaccharide in the biosphere. It has been estimated that 500 million tons of such materials could be annually available from the residues of major crops (Detroy, 1981). Effective extraction by enzymatic and microbiological processes of these materials is of great interest. Hemicellulase and endoxylanase enzymes have been extensively studied, since they hydrolyze polysaccharides in the pulp of woods (He et al.,1993).An attractive application of this hydrolysis process is the removal of xylan from wood pulp for manufacturing of dissolved pulp.

Recently, the interest in cellulase-free xylanases has focused on pulping and bleaching processes, in which chlorine (Cl2) and hypochlorite (ClO2) for bioleaching can be reduced (Viikari et al., 1991a; Kruss and Koljonen, M. 1991; Kruss et al. 1991). Naturally occurring microbial strains capable of secreting xylanases free of cellulase activity would be attractive for such applications. The most important enzyme needed for enhancing the bleaching of pulp is endo-B-xylanase (Kantalinen et al.,1988; Ludwig and Strunk, 1997; Viikari et al; 1991b). Xylanases enhance the cleaving of re precipitated xylan formed on the outer surfaces of the cellulose fibers after pulping (Kantelinen et al.,1991). This causes increased permeability of the

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Table 1. The chemical composition of analysis of papyrus and cotton stalk pulp

Material characterization	Papyrus pulp wt%	Cotton stalk pulp wt%
Humidity	7.9	10.3
Ash	0.9	1.0
Hollocellulose	88.0	90.1
Cellulose	66.2	70.0
Hemicellulose	22.1	23.1
Klason Lignin	24.5	27.0

pulp fibers to the bleaching chemicals and allows the passage of larger fragments of residual lignin out of the pulp.

Xylanases have been isolated from a wide range of microorganisms including fungi, actinomycetes and eubacteria. Actinomycetes enzymes, which are thermo stable, are of particular interest. Most actinomycete strains secreting high activity xylanases free of substantial cellulase activity are thermo tolerant: Streptomyces sp (Keskar et al.,1989). Saccharo monospora (Roberts et al.,1990) and Streptomyces roseiscleroticus (Grabski et al.,1991).Treatment with xylanases can improve the chemical extraction of lignin from pulp. This would lead to a significant reduction in the amount of chemicals required for bleaching and, hence, in the levels of toxic chlorine compounds released into the environment.

The present paper deals with the production of xylanase enzymes from various xylan assimilating Streptomycetes strains as potent producers of xylanase. In addition, Effect of EDTA has been studied on xylanase. Enzyme IR, X-ray and electro microscope of pulps were also investigated.

MATERIAL AND METHODS

Strain isolation

The cultures were isolated from a soil sample containing decomposed rice straw pulp detritus on a basal medium containing (g/l: 2.0 KNO3, 1.0 K2HPO4, 0.5 MgSO4, 3.0 CaCO3, 0.01 FeSO4 and 20.0 agar). Plates were incubated at 28ºC for seven days and single colonies were evaluated for growth. Streptomycetes colonies were picked followed by purification and preliminary evaluation of the isolates in shake flasks. Two of the strains, which showed high activity of xylanase production.

Characterisation of the two selected isolates

Characterization of the active isolates of Streptomycetes was done through polyphonic characterization. Morphological, physiological, chemo taxonomical and molecular identification were carried out according to various methods (Szabo et al.,1975;Williams et al.,1983; Holt et al.,1989 ; Ludwig and Strunk1997).

Culture media and conditions

The same previous basal medium was used. The pH was adjusted to 7.0-7.2 and the pulp substrate (2% w/v) was added prior to sterilization by autoclave at 121ºC for 15 min. Shake flask cultivations were carried out with 50 ml of medium in 250 ml flasks at 28oC and shaking at 200 rpm/min. Cultivation was done for 5 days after inoculation of 5 ml of a 5-day culture prepared on starch nitrate medium

Raw material

Papyrus (Cyperus papyrus) and cotton stalks were the raw material used in this work .It was cut into smaller pieces, then dried in an oven at 440 and packed in sealed polyethylene bags until required .Pulping was done in an heated autoclave (two revolution per minute)using 25% NaOH concentration based on the dry weight of raw material Time taken for pulping was 1.5h.liqo ratio 1:6and the pulping temperature 170CThe pulps were analyzed for hollocellulose (0)alpha cellulose ,hemicelluloses, klason lignin according to the relevant ASTM or Tappi standard methods The results are given in Table (1)

Treatment of the pulp with EDTA

Pulp was impregnated in water, was added as fine powder and the bulk was beaten up to SºR in a Jorko mill beaker at 150 r.p.m. measurements.

Table 2. Xylanase production by Streptomyces species growing on pulp at 2% w/v after 5 days

Maximum xylanase activity (u/ml) Material characterization	*Strptomyces rochei*	Streptomyces chromofucus
untreated cotton stalk pulp	13.2	19.3
treated cotton stalk pulp by EDTA	33.3	43.0
untreated papyrus pulp	9.3	13.2
treated papyrus by EDTA	17.2	25.7

Table 3. Effect of EDTA and Xylanase enzyme on the properties of pulp

Material	Klason Lignin	Brightness%	Kappa no.
untreated papyrus pulp	24.5	9.0	49.0
treated papyrus by *Strep.chromofucus*	11.2	16.9	22.4
untreated cotton stalk pulp	27.0	14.1	54.0
treated cotton stalk pulp by *Strept.rochei*	16.4	10.4	32.8
treated cotton stalk pulp by *Streptomyces chromofuscus.*	15.1	9.5	30.2

Pulp properties

Kappa number, degree of brightness and degree of polymerization were carried out according to the method of (Casey, 1980). Kappa number is used to describe the degree of delignification obtained in the chemical and/or enzymatic process. Degree of brightness (%), which means the whiteness of the tested paper, was measured by using a Hunter Lab color/Difference Meter D25-2 at wavelength 457 nm. Degree of polymerization (DP) is a function of average length of the cellulose chains and of fiber length. It is one of the significant factors of cellulose sample strength.

Infrared Spectra

The infrared spectra for treated and untreated cotton stalk is measured by using apparatus Jocko FTIR spectrophotometer, Japan. The samples were measured as KBr discs.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) of fractured surfaces of composite test specimens were conducted on JEDL JEM-100 S electron microscope using the gold.

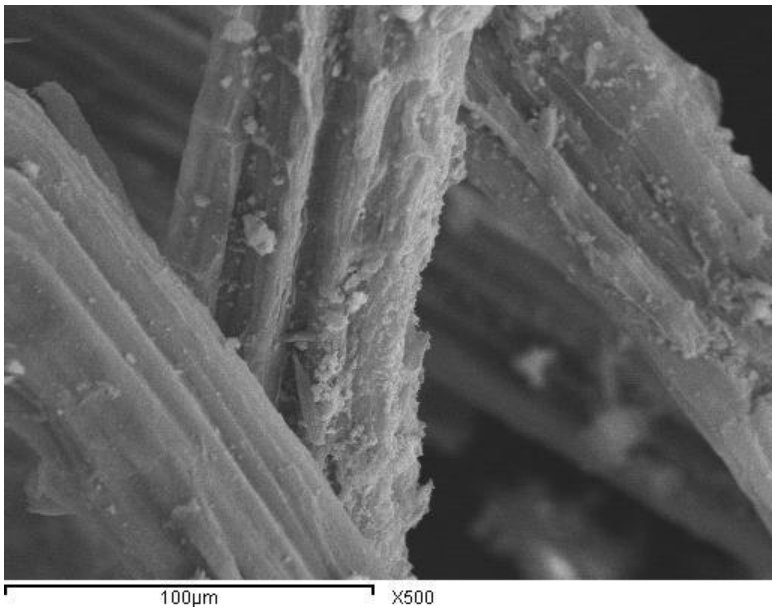
X-Ray Diffraction Method

The crystalline of samples was measured by X-ray diffraction (Bruker D8 ADVANCE, a Cu K α target with a nickel filter was used). The powder samples were pressed into discs in a special holder. The samples were scanned for a range of 20 from 10t.

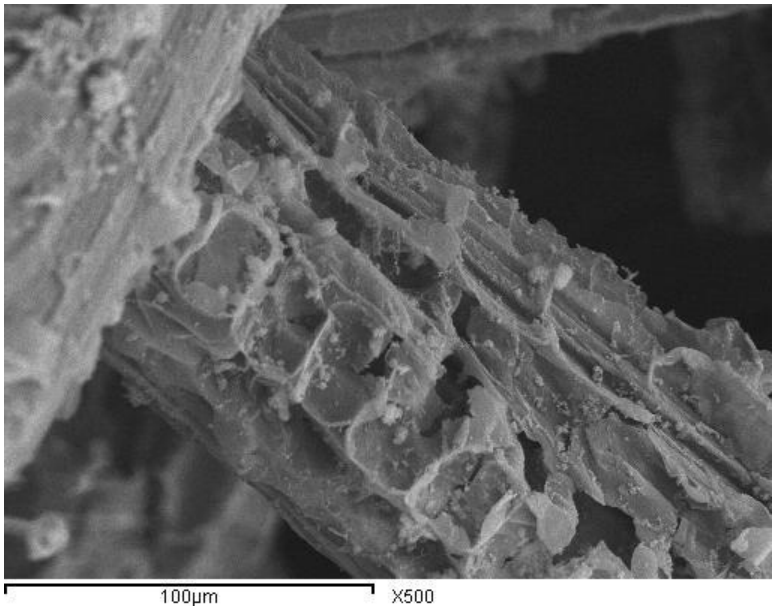
RESULTS AND DISCUSSION

Strain characterizations

Twenty Streptomyces isolates from Egyptian soils were screened for their xylanolytic activity. The most two



(a)



(b)

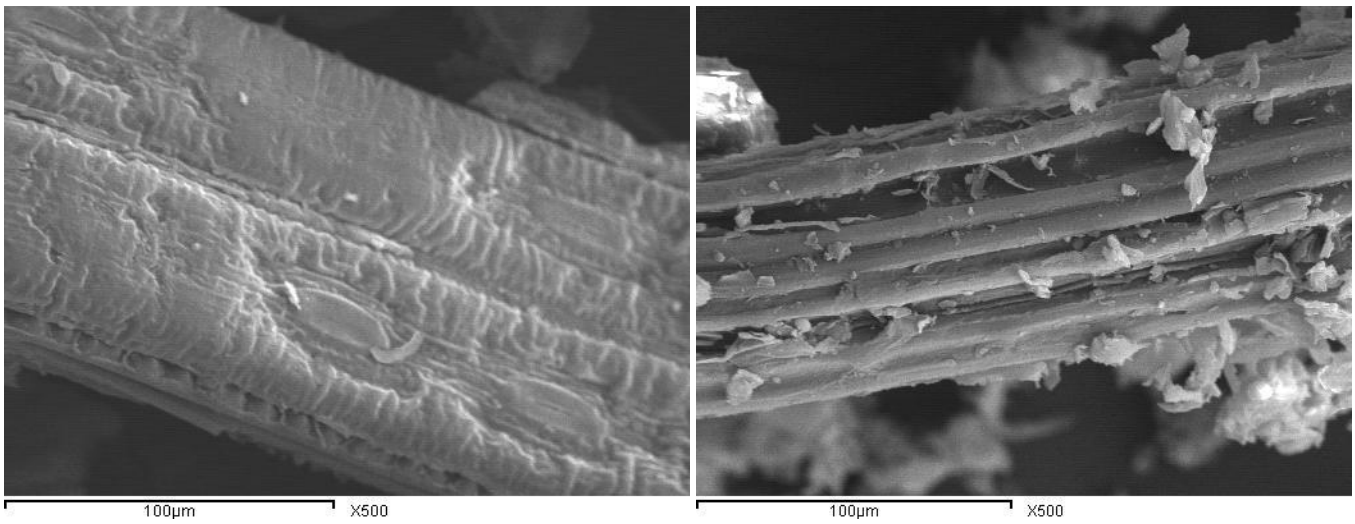
Figure 1. Scanning electron microscope (SEM) of (a) treated papyrus with *Streptomyces rochei*+ EDTA (b) control

active isolates have been taxonomically characterized. Based on the polyphonic characterization (morphological, physiological, chemo taxonomical and 16S r DNA sequences), the selected two isolates could be identified as Streptomyces rochei and Streptomyces chromofuscus.

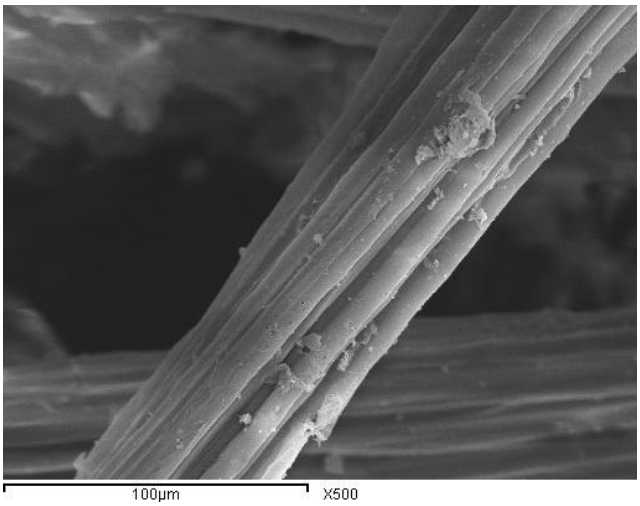
Xylanase production by Streptomyces spp.

Xylanase production using Sterptomyces rochei and Streptomyces chromofuscus was improved with untreated and treated different pulps as substrate. As shown in Table 1, xylanase was produced on treated pulp with a maximum activity of 43.01 u/ml. Streptomyces chromofuscus exhibited a higher xylanase activity using either untreated or treated pulp as substrate rather than Sterptomyces rochei (Table 2).

Cellulase activity was not detected in the culture supernatants of both cases. The activates of 13.2 or 19.3u/ml on untreated cotton stalk pulp to 33.3 in case of Streptomyces rochi and 43.0 in case of Sreptomyces chromofucus. Also the activates of 9.3or 13.2u/ml on untreated papyrus pulp to 17.2 in case of Streptomyces rochei and 25.7 in case of Sreptomyces chromofucus of were comparable or slightly lower than those reported for most other mesospheric actinomycetes that produce cellulase-free (Mawhinney et al., 2000).



(c) **(d)**



(e)

Figure 2. Electron microscope graphs of (c) treated cotton stalk pulps by *Streptomycses rochei* + EDTA (d) control(e) treated cotton stalk pulps treated *by Streptomyces chromofucus* +EDTA

It was observed presence of EDTA increased xylanase activity. Xylanase treatment improves accessibility of bleaching chemicals to pulp and decreasing diffusion resistance to outward movement of degraded lignin fragments from cell wall.

Effect of EDTA on Kappa number and Brightness of treated pulps

It is clear that xylanases may break down lignin-1bonds, improving the extractability of soluble lignin (Viikari et al.,1996) Alternatively, xylanase treatment improves accessibility of bleaching chemicals to the pulps, decreasing diffusion resistance of the degraded lignin fragments allowing lignin removal from the cell wall (Rifaat, et al., 2005).As a result pulps treated with xylanase show lower kappa number ,higher brightness and lower percentage of lignin than control(b)It is clear from table 3 that kappa number of papyrus pulp decreased from and 49 to 22.4 Brightness rose from 9% to 16.9% Lignin% also decreased from 24.5 % to 11.2% in case of treated with Streptomyces chromofuscus but Streptomyces rochei did not give good result or slightly difference than control. But in case of cotton stalk pulp Streptomyces chromofuscus much better than Streptomyces rochei. It was found that kappa number decreased from 54in case of control to 32.8. Streptomyces rochei. but 30.2 in case of Streptomyces chromofuscusyces Also brightness was enhanced from14.1%in case of control to 10.4%and9.5% in case of treated with Streptomyces rochei and Streptomyces chromofuscus respectively. Lignin% was enhanced by treated by Streptomyces rochei from 27% to 16.4% and to 15.1% in case of Streptomyces chromofuscus. This evidence is in agreement with our previous work (Enas et al.,2011; Torres, et al., 2000)

Scanning Electro microscope (SEM)

In order to understand the effect of EDTA on xylanase production from Streptomyces on enhanced bleach ability, the fiber surface morphology of the treated and untreated pulps without and with both species were

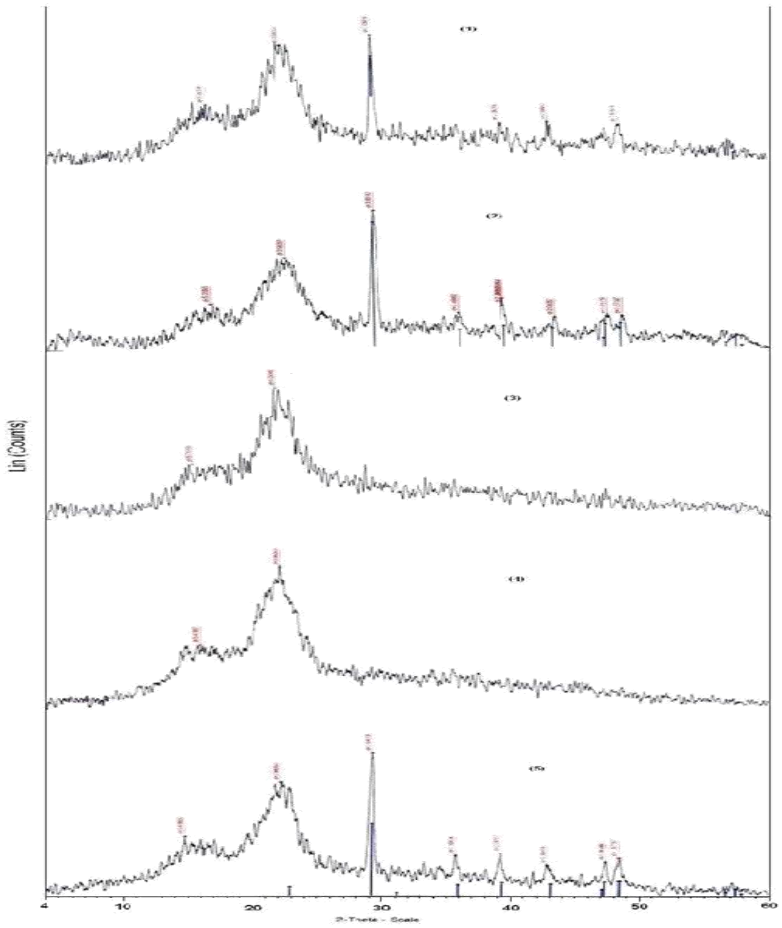


Figure 3. 2θ Diffraction angles

Table 4: Crystalline degree for treated and untreated pulps of Payprus and cotton stalk pulps

sample	a	b	c	d	e
Crystalline index	50.9%	51.5%	56.2%	55.7%	58.8%

studied by SEM. It was found that significant changes on the fiber surface of EDTA as a result of xylan hydrolysis, while no surface effect was observed in control pulps Fig.(1 and 2)comparing hard sheets made from untreated and treated pulps, the SEM of later shows that the EDTA has permeated into fibers pulp and has unified the bands strongly together fig.(1band2c,e).In addition ,the hard sheets made from pulp treated and untreated with Streptomyces rochei and Streptomyces chromofuscus reveals that fibers treated with EDTA exhibit cleaner surface apparently highly flexibility and conformability and fiber with Vemar kable peeling effect rather than control. Morphological changes such as holes, cracks, flakes, filaments and peeling are caused by enzyme treatment apparent compressibility and bending as compared to treated by Streptomyces this characteristic meets one of the required properties.

Examination of xylanase pretreated pulps revealed noticeable changed in the surface of the pulp in comparison with the control Fig (1 and 2) Morphological changes such as cracking and peeling of fiber surfaces were evident after xylanase and EDTA pretreatment This assisted in the surface modification but EDTA also help penetrated into pulps fibers allowing much improved xylan hydrolysis (Mawhinney et al., 2000).

X-ray

It is clear from X-ray diffract gram shown in Figure 3. It is possible to observe a major diffraction peak for 20

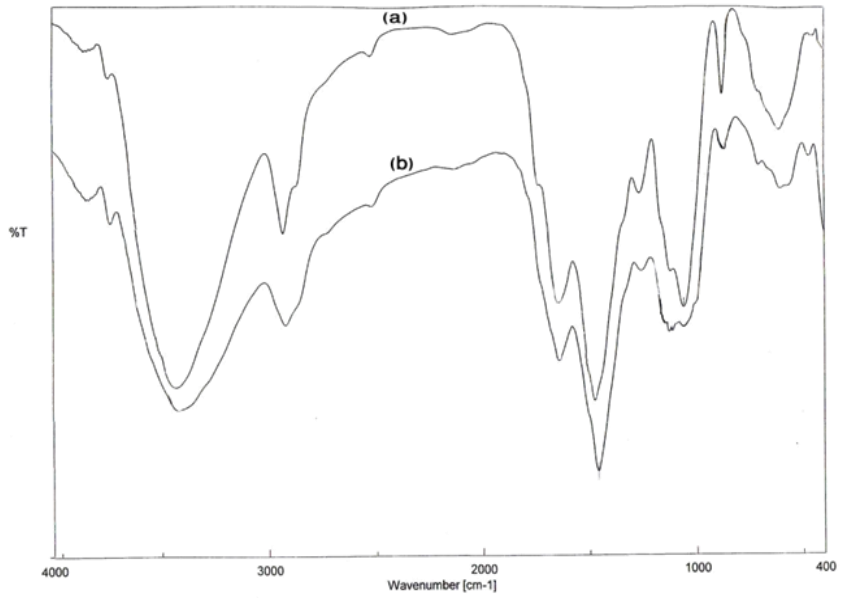


Figure 4. IR of (a) papyrus control (b) treated papyrus with Streptomyces chromofuscus.

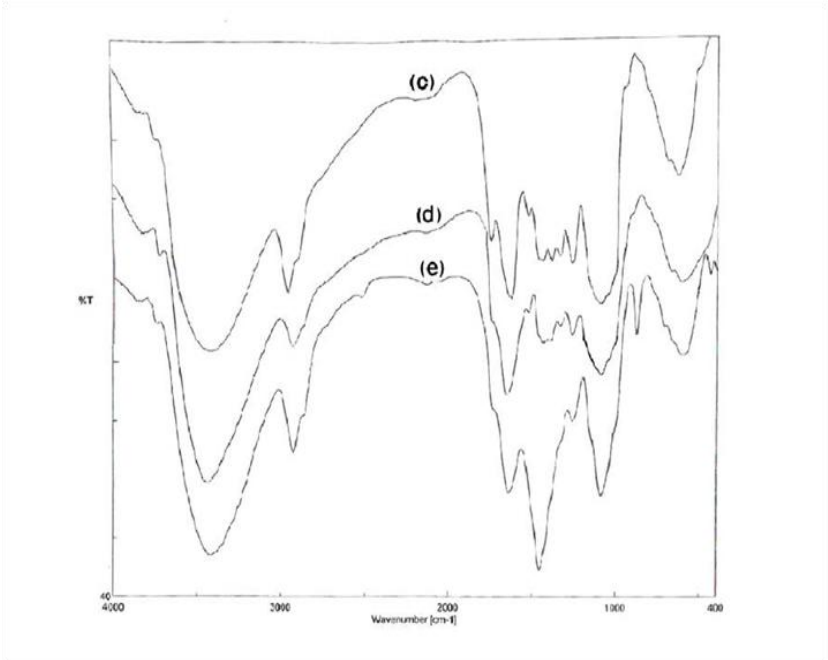


Figure 5. IR of (c) treated cotton with EDTA+ ***Streptomyces*** *rochei* (d)treated pulp with EDTA+ *Streptomyces chromofuscus.*

between 22° and 23° which corresponds to the cellulose fibers (002) crystallographic plane (Bansal et al.,2010)

In similar form to those reported for others lignocelluloses fibers. According to (Minopoulou et al., 2003). This method is to determine the lignocelluloses fiber crystallinity index provides simple and fast information. The crystallinity index (CI) was calculated as the ratio of the intensity differences in the peak positions at 16° and 22° according to equation:

According to this method the degree of the crystallinity values were calculated (Table 4) X-ray diffraction was used as an alternative method to determine the crystallinity of the material through the relative peak intensities. As shown from table(4) and fig (3), it can be concluded that the crystalline index of the samples from 1 to 5 behave as follows, treated pulps much more crystallite than untreated Incase of cotton stalk pulps crystalinity much more better than papyrus pulps It was found that crystallinity reached 58.8 in case of with

Streptomyces chromofuscus. much more better than Streptomyces rochei which reached 56.2%It was observed that the enzymes have a great role for increase the crystallinity of pulps.

FT-IR spectroscopy

It was clear from IR spectra of treated and untreated cotton stalk and papyrus pulps that the enzyme treatment had removed substantial amount of xylan from the fibers, while the cellulose appeared to be relatively unchanged.

From fig 4 and 5 it was found that the characteristic cellulose peaks around 1000-2000 cm−1(Bouchard and Douek., 1993) The relative high absorbance at 1045-1050cm−1 ,and the bands at 1460,1250,811cm−1 indicated the presence of some hemicelluloses ,while the weak absorption band at 1512cm−1 shows that only a small amount of lignin was still present (Wong et al.,1996). The major difference was sawn to be around 1045-1055cm−1 ,which corresponds to the native xylan spectra at 1045-1058cm−1 ((Wong et al.,1996)These results confirmed that the total amount of xylan in the sample had been reduced by EDTA and enzyme treatment The.895cm−1 band which is characteristic for B -linkages, especially in hemicelluloses also reduced. However, the band 811cm−1 which is characteristic of galacto-glucomannan(Fengel,1992) This is a good agreement with the chemical analysis of the pulps Table 3.

CONCLUSION

Xylanase production using two types of Sreptomyces *S. rochei* and S. c*hromofuscus* was improved with untreated and treated different pulps as substrate. Xylanase was produced on treated pulp with a maximum activity of 43.01 u/ml. S. *chromofuscus* exhibited a higher xylanase activity using either untreated or treated pulp as substrate rather than S. *rochei.* Xylanase treatment gives lower kappa number, higher brightness and lower percentage of lignin than control kappa number of papyrus pulp decreased from and 49 to 22.4 Brightness rose from 9% to 16.9% Lignin% also decreased from 24.5 % to 11.2% in case of treated with S*. chromofuscus*. But in case of cotton stalk pulp S. *chromofuscus* much better than S. *rochei.* Also brightness was enhanced. It was noticed that from electro microscopy xylanase pretreated pulps revealed noticeable changed in the surface of the pulp in comparison with the control such as cracking and peeling of fiber surfaces were evident after xylanase and EDTA pretreatment. From x-ray studies it was observed that the enzymes have a great role for increase the crystallinity of pulps. From IR spectra the major difference was sawn to be around 1045-1055cm−1, which corresponds to the native xylan spectra at 1045-1058cm−1 which showed that the total amount of xylan in the sample had been reduced by EDTA and enzyme treatment.

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