Characterization Properties of Rice Straw Pulp Treated with Streptomyces Species and Titanium Dioxide

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The number of possible application of microbial xylanases in the pulp and paper industry is gradually increasing and several are approaching commercial use. This industry needs xylanase, which is free of cellulose. Newly isolated Streptomycetes, which produce cellulase free-xylanase and are easily grown using a low-cost agriculture waste substrate, were investigated Twenty Streptomyces isolate from Egyptian soils were screened for cellulase-free xylanase activity. The most two actives have been identified as Streptomyces albus and Streptomyces chromofuscu. Their maximum activity were 13.25, 19.31 and 32.53, 43.01 on untreated rice straw pulp and treated pulp with TiO_2 on both Streptomyces species respectively.

IR, X-ray and electrical resistivity were studied from rice straw treated with 20% TiO_2 and then exposed to Streptomyces species.

Keywords : Rice straw pulp; Streptomyces Species; IR spectra; X-ray diffraction; electrical resistivity,

INTRODUCTION

Rice straw is one of the main cereal straws and is produced in large quantities world-wide every year. In developing countries, these large quantities of fibrous crop residues are currently under-utilized either as raw material for paper making, or as potential animal feed sources. However, such straw is considered to be agricultural waste in developed countries since it cannot be converted into valuable products[1]. As we attempt to reduce the adverse impact on the environment and to use this renewable biomass to produce various chemicals, the development of effective technologies for utilization of straw is considered to be both important and significant.

The number of possible application of microbial xylanases in the pulp and paper industry is gradually increasing and several are approaching commercial use. This industry needs xylanase, which is free of cellulase. Newly isolated streptomycetes, which produce cellulase free-xylanase and are easily grown using a low-cost agriculture waste substrate, were investigated [2]. Twenty Streptomyces isolates from Egyptian soils were screened for cellulase-free xylanase activity. The most two actives have been identified as Streptomyces albus and Streptomyces chromofuscus. Their maximum activity were 13.25, 19.31 and 32.53, 43.01 on untreated rice straw pulp and treated pulp with TiO_2 on both Streptomyces species respectively. Xylanase inhance the cleaving of reprecipatated xylan formed on outer surfaces of the cellulose fibers after pulping. This causes increased permeability of the pulp fibers to the bleaching chemicals and allows the passage of larger fragments of residual lignin out of the pulp [3].

Filler materials are usually incorporated into paper to improve various properties [4]. Calcium carbonate, gypsum and titanium dioxide are used as mineral fillers because they provide special properties.

Titanium dioxide [5-8] is the most widely used white pigment, with versatile characteristics such as a high refractive index, stability and non-toxicity. It is available in two crystalline forms, anatase and rutile. The latter variety is thermally more stable and resistant to chemical attack and opacifies better than the anatase grade. Therefore, anatase is used largely in paints for interior and rutile for exterior applications. Ultra fine TiO_2 offers completely new options in protecting wood coatings.

Xylanase was also shown to increase fiber wall swelling and in turn increase the speed of diffusion through the walls[9]. Since it is believed that extraction of depolymerized lignin from pulp is a diffusion-limited process. Xylanase treatment ultimately improves the extraction of lignin from pulp [10]. Thus, for enzyme treated pulp, subsequent bleaching stages are more efficient, and higher brightness can be expected. Other work suggests that if lignin covalently bound to xylan was made smaller by enzyme use, it would be more easily extracted [11].

Another hypothesis that came from research was that xylanase enzymes catalyze the hydrolysis of xylan that has reprecipitated on the fibers during alkaline pulping [12]. Removal of this xylan was thought to remove a physical barrier preventing the extraction of residual lignin. However, recent work has shown that pulp prepared under conditions that prevent xylan reprecipitation also responds well to the xylanase bleach boosting effect [13]. Following the first application of xylanases in pulp bleaching, other studies have demonstrated the benefits of enzymes in the bleaching of softwood [14-15] and hardwood pulp [16].

Xylanases have been isolated from a wide range of microorganisms including fungi, actinomycetes and bacteria. Actinomycete enzymes, which are thermostable, are of particular interest, as they play an important role in the carbon cycle. Other actinomycete strains secreting high activity xylanases free of substantial cellulase activity include thermotolerant: Streptomyces [17], Saccharomonospora [18] and Streptomyces roseiseleroticus [19]. 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 aim of our work to investigate chemical, physical & electrical studies of rice straw pulp untreated & treated by 20% TiO_2 and then exposed to two different strains of Streptomyces species.

EXPERIMENTAL

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 sterllisation by autoclave at 121°C for 15 min. Shake flask cultivations were carried out with 50 ml of medium in 250 ml flasks at 28°C and shaking at 200 rpm/min. Cultivation were done for 5 days after inoculation of 5 ml of a 5-day culture prepared on starch nitrate medium.

Xylanase assay

The activity of xylanase was measured by following the release of reducing sugars from soluble xylan. The reaction mixture consisted of 5 ml of xylan solution (3% as anhydroxylose), 4 ml of 0.1 M phosphate buffer (pH 6.5) and 1 ml of enzyme solution. The reaction was run at 40°C for 30 min. Increase of reducing sugars in the mixture was determined as xylose by the method of Somogyi [20]. One unit of xylanase is defined as the amount of enzyme, which is capable of liberating IMM equivalent of xylose in one minute. Cellulase activity was also determined using the same procedure in addition to 5 mg/ml cellulose.

Pulping

Rice straw was cut into 3-4 cm then extracted by a mixture of methanol and benzyene (1: 1) for 6 hour to remove waxes and resins. The residual material was air dried. Soft wood soda pulps were prepared by cooking these dried materials under the following conditions: total alkali 12% and cooking temperature 160°C for 1 hour.

Treatment of the pulp with TiO_2

Pulp was impregnated in water. TiO_2 was added as fine powder and the bulk was beaten up to So °SR in a Jorko mill beaker at 150 r.p.m.

Pulp properties

Kappa number, degree of brightness and degree of polymerization were carried out according to the method of [21]. 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 colour/Difference Meter D25-2 at wavelength 457 nm. Degree of polymarisation (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.

Infra-red spectra

The infra-red spectra for wood pulp and the prepared papers were obtained using a Jocco FT/IR 430E spectrophotometer Japan. The samples were measured as KBr disces.

Crystallinity Measurements

The crystallinity of cellulose was measured by X-ray diffraction, [Bruker D8 ADVANCE, a Cu Kα target with a nickel filter was used].

The cellulose powder was pressed into discs in a special holder. The samples were scanned for a range of 20 from 10° to 30°. The following X-ray crystallinity index DC, as proposed by Segal et al [22] was employed

$$DC = [I_{22} - I_{18}] / I_{22} \qquad [22]$$

Where I_{22} is the intensity of the 22°C peak (at about 20 = 22°), which corresponds to the crystalline fraction of cellulose. I_{18} is the intensity at about 20 = 18° Which corresponds to the amorphous region which produce a wide band [23].

Resistivity Measurements

The samples in the from of discs of diameter 12.5mm, prepared by pressing the sample powder of mass 0.250g in a mold, under pressure of 750 Mpa. Electrical resistivity was measured using a specially designed cell holder, in which the sample was fixed between two polished copper electrodes, so that to insure good electrical contact. A high impedance digital Keithley electrometer, model 6517 A, was used. The sample resistance was measured at room temperature (30°C). Each sample was then annealed for 10 min at 50, 80, 100 and 120°C and the resistance is re-measure.

RESULTS AND DISCUSSION

Strain characterisation

Twenty Streptomyces isolates from Egyptian soils were screened for their xylanolytic activity. The most two active isolates have been taxonomically characterized. Based on the polyphasic characterization (morphological, physiological, chemotaxonomical and 16S rDNA sequences), the selected two isolates could be identified as Streptomyces albus (1) and Streptomyces Chromofuscus (2).

Xylanase production by Streptomyces species

Xylanase production using Streptomyces albus and Streptomyces chromofuscus was improved in the untreated and treated rice straw pulp as substrate. As shown in Table (1), xylanase was produced from treated pulp with a maximum activity of 43.01 u/ml. Streptomyces chromofuscus exhibit a high xylanase activity using either untreated or treated pulp as substrate rather than Streptomyces albus Table 1. Cellulase activity was not detected in the culture supernatants of both cases. The activity of 19.31 or 13.25 u/ml were comparable or slightly lower than that reported for most other

Table 1: Xylanase production by Streptomyces species growing on pulp at 2% w/v after 5 days

Substrate	Maximum xylanase activity (u/ml)	
	Streptomyces albus	Streptomyces chromofuscus
Untreated pulp	13.25	19.31
Treated pulp with TiO_2	32.53	43.01

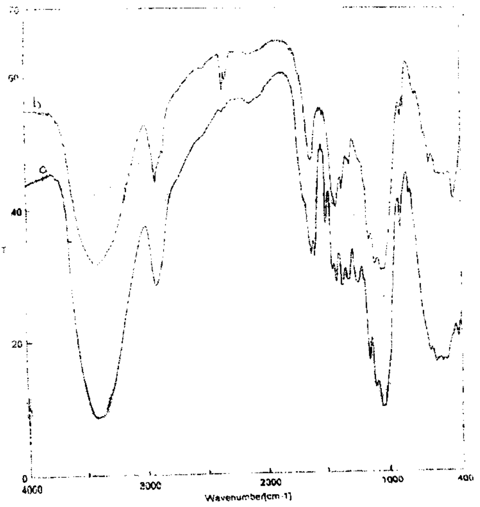

Figure 1 :

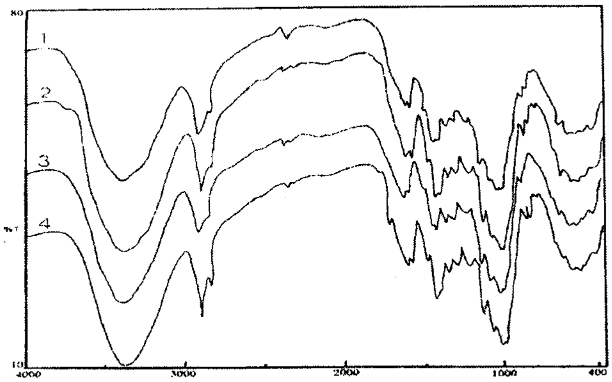

Figure 2 :

mesophilic actinomycetes that produce cellulase-free xylanase [24] reported 12 u/ml of xylanase in 5 days culture of Streptomyces albus. Such slight levels of cellulase-free xylanase products from treated crude agricultural waste has been reported. Growth of xylanase was inducible by treated rice straw pulp in both Streptomyces species.

Hydrolysis of the pulp

It is clear from Table (1) that hydrolysis of rice straw soft wood pulp with xylanase liberated reducing sugar from Streptomyces chromofuscus (1) more effective than Streptomyces albus (2). The amount of reduced sugars greatly increased in Streptomyces albus from (132 to 140) mg/ml and from (149 to 170) mg/ml in Streptomyces chromofuscus. Moreover, pulp treated with TiO_2 enhance hydrolysis. i.e. increasing reduced sugar and brightness which is in accordance with the results of [25]. This means that xylanase treatment improved bleachability and did not affect the quality of the pulp. Such results are better than those of previously reported enzymes [26&27] . This implies that the coloured substance (s) in the crude pulp are probably bound to cellulose by xylan rather than by mannan. The degree of brightness in case of Streptomyces albus increased from 45.1 to 47.2 (untreated) and to 49.1 % (treated). Remarkable increase in brightness is observed in case of Streptomyces chromofuscus from 45.1 to 50.2 (untreated) and to 54.2% (treated). Kappa no. in treated pulp decreased 4 points in case of Streptomyces albus and 7 points in case of Streptomyces chromofuscus.

The prepared enzyme had no cellulose activity and showing a noticeable increase in the degree of polymerization (DP). It may be probably due to selective degradation of the low molecular mass fractions of xylan [28]. It is clear that DP increase from 960 to 970 to 990 and from 960 to 995 to 1110 in the untreated and treated samples of Streptomyces albus and Streptomyces chromofuscus respectively (Table 2). Concerning reduced sugar, the treated samples especially Streptomyces chromofuscus reached 170 mg/ml.

IR spectra

The FTIR spectra of the soft wood pulp are treated and untreated with streptomyces species illustrated in Fig. (1&2).

The broad strong absorption band centered at about 3440 cm^{-1} is due to hydrogen bond OH stretching vibrations. The band at about 2920 cm^{-1} is due to CH stretching vibrations. Hon and Chang [29] attributed the band at about 1640 cm^{-1} in a spectrum of wood pulp to C=O

Table 2 : Hydrolysis of soft wood rice straw pulp treated and untreated by Streptomyces species

Strain	Samples	Degree of polymerization	Kappa No.	Degree of brightness	Reduced sugar mg/ml
Streptomyces Albus	Blank	960	17.3	45.1	-
	Untreated	970	15.2	47.2	132
	Treated	990	13.2	49.1	140
Streptomyces C hromofuscus	Untreated	995	12.5	50.2	149
	Treated	1110	10.5	54.2	170

stretching of carboxyl and acetyl group in the H-O methyl glucurono acetyl xylan components of hemicellulose. It is evident that the band at 1625 cm-l in the spectra originates from its hemicellulose components. Liang etal [30] noted that in wood pulp the band near 1375 cm^{-1} is due to CH$_2$ deformation maximum near 1245 cm^{-1} is similar to that in the spectra of wood pulp corresponding to the C-O link in R - O - C- CH$_3$ gorup xylan. They further pointed out that lignin and cellulose might have also contribute to this broad band at 1240 cm^{-1} in the spectra of Jute fiber.

The band about 1420 cm^{-1} is ascribed to CH$_2$ sissoring motion in cellulose [29-31], while the bands near 1380 cm^{-1}, 1330 and 1320 cm^{-1} are due to CH deformation, OH in plane bending and CH$_2$ Wagging respectively, cellulose and xylan, might contribute to these bonds Liong[33,35], assigned the band in lignin and xylan, as well as CH$_2$ bending in xylan Sao et al [31] reported that both lignin and hemicellulose contribute to the band at 1375 cm^{-1} in the spectrum of jute.

The spectra of softwood pulp between the region 1200 cm^{-1}-1400 cm^{-1} exhibit a broad weak band it 1250 cm^{-1}. This band appears to be a doublet having maxima near 1247 cm^{-1} and 1320 cm^{-1}. In cellulose these two bands were possibly assigned to C-O-C band in cellulose chain and OH deformation respectively. Sao *et al* [31] stated that the broad medium band having at 1160 cm^{-1} to stretching or OH. bending of the C-OH group. The two bands at 1160 and 1050 cm^{-1} are both attributed to cellulose and hemicellulose [30]. The weak band appearing at about 900 cm^{-1} is characteristic of β-linkage [32].

The above mentioned structural assignments of the absorption bands lead to the conclusion that IR spectra of soft wood pulp which is a naturally occurring polymer is due to the variable changing relative to each other. The pronounced effect of the enzyme is observed in the case of sample (4).

The band at about 895 cm^{-1} which is characteristic of β-linkage, especially in hemicelluloses become less intense after enzyme treatment. The band at 1635-1640 cm^{-1} which was attributed to the absorbed water bending vibration become more intense and splited into two bands. At 1635 cm^{-1} and 1640 cm^{-1}. The strong band which is central at 3400 cm^{-1} is due to OH vibration of intermolecular H-bonds, showed no observable changes on enzyme treatment. From the above mentioned results one can come to the conclusion that the soft wood pulp which has subjected to enzymatic treatment assumed some changes in its structural properties particularly, in its

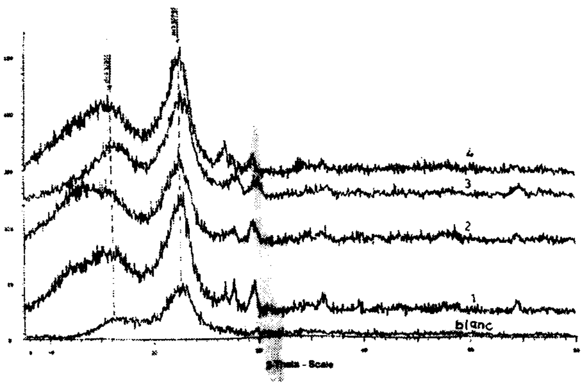

Figure 3 :

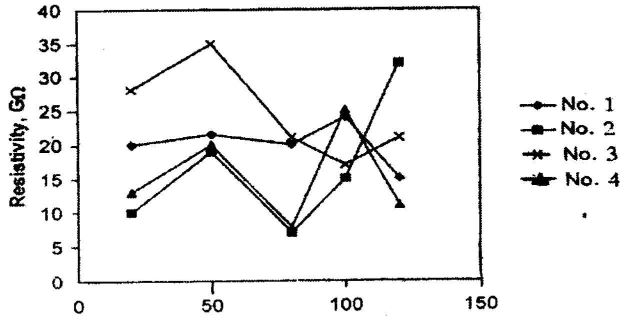

Figure 4 : Temperature, °C

crystallinity. Also the enzyme treatment caused considerable hydrolysis of xylan fraction the combined absorption of individual components namely cellulose, hemicellulose and lignin. Careful examination of the spectra of samples under investigation indicates the following :

Enzymatic treatments have some effects on the structured of soft wood pulp. The FTIR spectra of untreated samples show characteristic absorption of nearly bands equal hights at 1430, 1375 and 1320 cm^{-1}.

The enzyme treatment caused significant changes in the spectral feature of this region.

Table 3 : Crystallinity variation of rice straw pulp treated with 20% TiO_2 and exposed to Streptomyces and Inzimes.

Sample No.	(CD) crystallinity Index
1	0.51
2	0.48
3	0.50
4	0.42

For example the intensity of the three bands intermolecular H-bonds, showed no observable changes on enzyme treatment. From the above mentioned results one can come to the conclusion that the soft wood pulp which has subjected to enzymatic treatment assumed some changes in its structural properties particularly, in its crystallinity. Also the enzyme treatment caused considerable hydrolysis of xylan fraction.

Crystallinity index

As it can be seen from the table (3), The D.C. calculated from this Fig. (3) for the rice straw pulp untreated & treated with TiO_2 20% concentration was treated by enzymes Streptomyces species. This showed that the highest value (D.C.) 0.51 at sample No. 1 followed by the sample No.3 which has D.C. value 0.50 and that with sample No. 2 which has a value of 0.48. The sample No.4 has the lowest D. C. value (0.42) as shown from Fig. (3) and the Table (3).

The cellulose lines of d=3.92826 at about 22°C (2θ) and d = 5.2005) at about 2θ 18° are clearly appeared to vary in intensities by the variation of enzymes treatment which contains 20% TiO_2 concentration shown in the next table.

Table 4 : Intensily of streptomyces species (18° and 22°) lines with the variation of enzymes.

	Sample no. (1)	no.(2)	no.(3)	no.(4)
Line d = 5.2005 of 2θ -18	49	100	90	120
Line d=3. 92826 at 2θ =22	100	160	174	208

Table 5 : Electrical resistivity of rice straw pulp treated with 20% TiO_2 and exposed to streptomyces species.

resistivity / Temperature °C	Samples			
	No.1	No.2	No.3	No.4
20	20	10	28	13
50	21.5	19	35	20
80	20	7	21	7.8
100	24	15	17	25
120	15	32	21	11

It can be concluded that the crystallinity index of the samples from 1 to 4 behave as follows. First sample is the highest while the fourth sample was the lowest. Also, the intensities strength is the highest intensity strengths at 2θ 18° and 22° for the sample (4).

Electrical resistively

Table 5, Fig.4 shows the change in resistivity with the variation of temperature. Samples with concentration of 20% TiO_2 treated by Streptomyces species and heat treated for 10 minutes of 50°C, 80°, 100°, and 120°C.

- At the fig the first sample (no. 1) has the maximum resistivity at 100°C and then decreased at 120°C.
- The second sample has increased at 50°C and then highest increased at 120°C.
- The third sample has increased at 50°C and then decreased in the remain temperature.
- The fourth sample has increased at 50°C and then decreased at 80°C.

All samples are increased in resistivity with increasing temperature until it reaches at 50°C then the resistivity begin to decrease till it reached its minimum value at 80°C. After that each had been acting randomly.

The first sample has no effect in resistivity till 80°C then at 100°C it increased smoothly then decreased at 120°C. So it can be concluded that the TiO_2 has its best effect at 80°C.

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Captian of the Fig.

Fig. (1). IR Spectra of (a) rice straw pulp (b) treated with 20% TiO_2.

Fig. (2). IR spectra of rice straw pulp.

(1) exposed to Streptomyces albus.

(2) Exposed to Streptomyces albus treated with 20%TiO_2.

(3) Exposed to Streptomyces chromofuscus.

(4) Exposed to Streptomyces chromofuscus treated with TiO_2.

Fig. (3). X-Ray spectra for Blank

(1) exposed to Streptomyces albus.

(2) Exposed to Streptomyces albus treated with 20%TiO_2.

(3) Exposed to Streptomyces chromofuscus.

(4) Exposed to Streptomyces chromofuscus treated with TiO_2.

Fig. (4). Resistivity for

(1) exposed to Streptomyces albus.

(2) Exposed to Streptomyces albus treated with 20% TiO_2.

(3) Exposed to Streptomyces chromofuscus.

(4) Exposed to Streptomyces chromofuscus treated with TiO_2.