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Investigation of Crystallinity Characterization of Bamboo Fibers Using Xylanase from *Aspergillus nidulans*

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ABSTRACT
The Crystallinity phenomenon refers to a crucial feature of lignocellulosic biomass due to its substantial impact on enzyme treatment. The X-ray diffraction analysis is applied to determine the parameters characteristic of the bamboo fibers such as crystallinity index, crystalline %, crystallite size as well as Z- value. Results of the X-ray diffraction analysis appeared maximum in treated bamboo fiber at temperature 40 ºC. They estimated the highest value in 57% of crystallinity index, 72% of crystalline %, 3.91nm of crystallite size and -34.6 of Z- value in treated bamboo fiber than untreated bamboo fiber. This increase could be attributed to the completely removal of amorphous non-cellulosic compounds by xylanase treatment, which would give better smoothing of the surface of bamboo fiber.

Keywords: Xylanase, crystallinity index, crystallite size, crystalline %, Bamboo fiber, Z- value.

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INTRODUCTION

Cellulose refers to a polymer of β-glucose with β-1-4 bonds between the units of glucose. There are two regions within cellulose. The crystalline region of cellulose constituted of highly ordered cellulose molecules reaching 2/3 of the whole cellulose, whereas within in the amorphous region contains less ordered molecules [1].

These crystalline regions and amorphous regions have been identified by X-ray diffraction analysis such as crystallinity index, crystalline %, crystallite size as well as Z- value. Bamboo fiber termed as one of the lignocelluloses producing plants which contains cellulose 73.8%, hemicellulose 12.5%, lignin 10.1%, and pectin 0.4% [2].

Bamboo fiber has some major disorders such as harsh prickly feel and poor drapeabity. Therefore, it is necessary to remove these disorders through biological processing by biotechnology termed xylanase treatment [3]. The enzymatic approach of fibers consider an environmental process due to enhancing of the characteristic of the fiber, their desirable surface as well as the soft handle [4].

Our study aimed to determine the characteristics of the X-ray diffraction of bamboo fibers using xylanase from *Aspergillus nidulans*.

Materials and Methjods

Enzyme treatment of the bamboo fibers

The material used in this work was raw bamboo fiber which was obtained from agricultural nursey in Iraq. The bamboo clum was chopped into small chips after removing the epidermis from bamboo and then chopped into small chips. After that their small chips were put in a hammer mill and then grounded, sieved using a 100µm sieve. The bamboo powder dried 72h in an oven at 70ºC and placed in a glass jar for further analysis. Dried bamboo powders weighing 200mg were immersed in test tubes containing 10ml (50mM, pH7.2) phosphate buffer and 10ml of 100% crude xylanase enzyme dosage. The tubes were incubated for six hours at 30°C, 40°C and 50°C. Test tubes were then boiled in a water bath for 5 min. to finish enzyme activity and cooled at room temperature. After that, the reducing sugars were determined in the incubation mixture as described in Ghose,(1987) [5]. After that, these bamboo fibers were air-dried and used as further study.

X-Ray Diffraction (XRD) studies

The structural characteristic of the raw bamboo fibers, as well as treated bamboo fibers, were achieved to measure the crystallinity degree by wide-angle of X-ray diffractogram. The powder fibers were examined between angles (2θ) 5° to 70° to obtain the equatorial reflections using X-ray diffractometer (XRD) Bruker AXS D8 with CuKα radiation (λ- 1.5418Å). The generator was utilized at 40KV, 30mA at a scanning level of 1.2°/ 1min.

The crystallinity index (C.I) was determined by the equation as follows (Sajithkumar *et al*., 2016) [6]:

$$C.I\% = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$

Where I_{200} the intensity of the peak from 200 lattice plane (2θ=22.4º) representing crystalline material while I_{am} the diffracted intensity peak at 2θ =18º indicating amorphous material of bamboo fibers.

The percent crystallinity of the raw and treated bamboo fibers of each temperature 30 ºC, 40 ºC, and 50 ºC was evaluated using the equation given below (Kaith and Kalia, 2008) [7]:

$$\text{Crystalline } \% = \frac{I_{22}}{I_{22} + I_{18}} \times 100$$

Where I_{22} and I_{18} are the 2θ scale of 22º and 18º representing the crystalline and amorphous intensities respectively.

The crystallite size of the fibers samples was evaluated using the following Scherrer formula (Han *et al*., 2014) [8]:

$$L = K\lambda / \beta \cos \theta$$

Where L, the crystallite size; K, the shape factor (0.94); λ, the X-ray wavelength used; B, the half-width maximum of the equatorial reflections and θ, Bragg angle related to the 200 plane.

The Z-discriminant (Z-value) analysis was achieved for the differentiation of cellulose whether I_α (monoclinic) or I_β (triclinic) of the crystalline cellulose structure in bamboo fibers was utilized (Wada and Okano., 2001) [9]. This analysis is determined by Equation (Poletto *et al*., 2012) [10]:

$$Z = 1693d_1 - 902d_2 - 594$$

where d_1 is the d-spacing of the peak (1–10); d_2 is the d-spacing of the peak (110), and $Z > 0$ corresponds to I_α ; whereas $Z < 0$ refers to the I_β dominant cellulose type (Wada and Okano ., 2001) [9].

Results and Discussion

The X-ray diffraction patterns of the untreated and treated bamboo fibers at different temperatures of 30 °C, 40 °C and 50 °C are illustrated in figures (A), (B), (C), (D), (E) and (F) respectively. The diffractograms were observed at the peak range between 13.1 and 15.4 2θ reflections attributed to the (11^-0) crystallographic plane, The peak range 14.8-15.99 2θ reflection is corresponding to the (110) crystallographic plane and the peak range between 21.7-22 2θ reflection belong to 200 crystallographic plane. These all crystallographic planes were indicated the appearance of cellulose I structure in the fibers, both before and after xylanase treatment.

The gradual removal of amorphous contents specially lignin and hemicelluloses altered the nature of fiber to extra crystalline that are indicated by these diffractograms, where the intensities of the crystalline planes 110^-, 110, 200 reflections were obtained to be maximized for the treated bamboo fibers. These alterations for each treatment attributed to the dismissal of these amorphous contents leading to the decline of the disordered regions.

In the temperature 40 °C of the treated bamboo fiber where the crystalline plane 200 of diffractogram is sharper compared to 30 °C and 50 °C which indicative of a higher crystallinity degree (i.e crystalline pattern of cellulose) in the structure of treated bamboo fiber as described in figure (D).

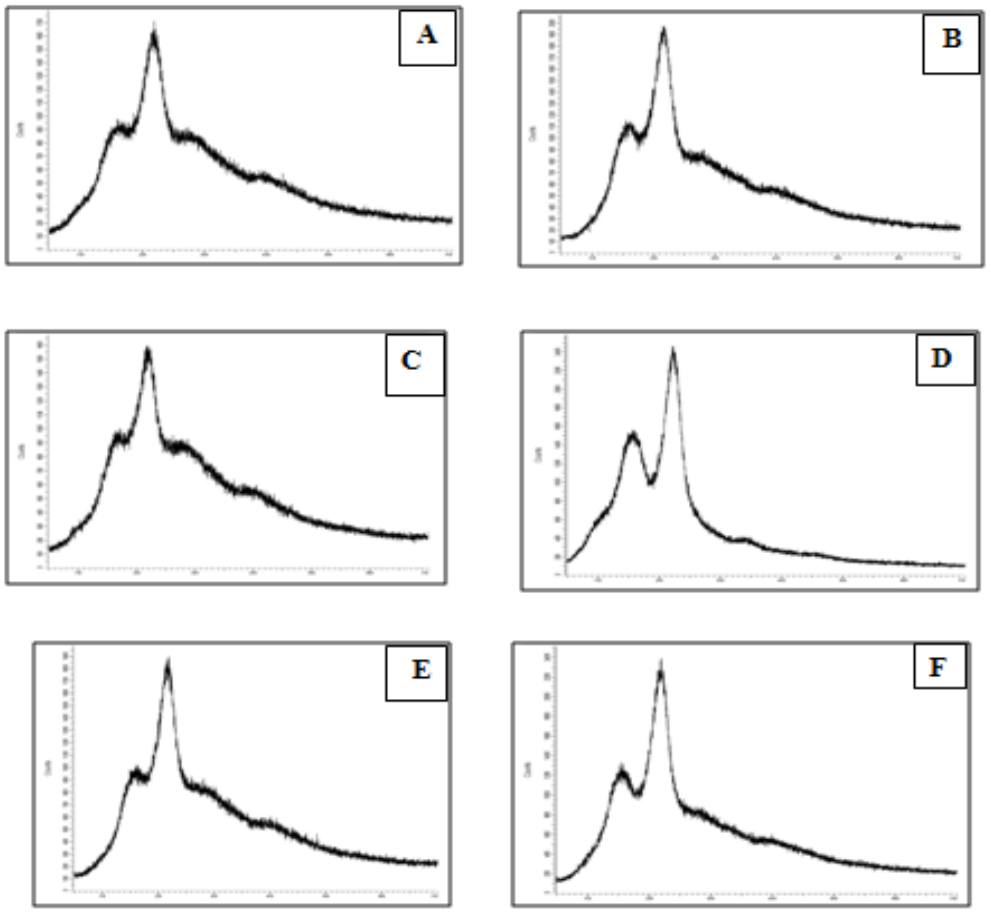

Figure1: X-ray diffractograms patterns of the untreated and treated bamboo fibers in the different temperatures 30 °C, 40 °C and 50 °C;

A. untreated bamboo fiber 30 °C; B. Treated bamboo fiber 30 °C;
C. untreated bamboo fiber 40 °C; D. Treated bamboo fiber 40 °C;
E. untreated bamboo fiber 50 °C; F. Treated bamboo fiber 50 °C.

The crystallinity index CI% of untreated and treated bamboo fibers were processed for semi-quantitative estimation of the amounts of amorphous and crystalline regions in cellulose structures by XRD analysis as shown in table (1). The temperature and concentration of the enzyme 100% had a significant effect on the crystallinity index of bamboo fibers. In the table (1) the crystallinity index of bamboo fiber was changed, depending on the temperatures 30 °C, 40 °C and 50 °C.

Under the treatment temperature 30 °C, the crystallinity index (CI) of treated bamboo fiber was slightly increased which reported 49% rather than untreated bamboo fiber which recorded 48%. This may be attributed to the

removal some of the amorphous non- cellulosic compound lignin by the xylanase treatment, which would allow better packing of the cellulosic chains.
In temperature 40 ° C, with the treatment of xylanase 100%, the lignin is removed and the crystallinity index goes on rising which observed to be 57 % compared to the raw bamboo fiber 41%. This may be due to the dismissal of lignin and hemicellulose which acts as a cementing substance and on delignificant, an ordered arrangement of the crystalline cellulose in the structure takes place. While in the temperature 50 °C, the improvement of the crystallinity index of treated bamboo fiber in comparison to the untreated bamboo fiber as reported 55 %, 51% respectively. This is consistent with the increasing crystallinity index, suggesting that the non-crystalline materials were decreased.

Table 1: Parameters obtained from the XRD analysis of the bamboo fiber before and after xylanase treatment 100% under different temperatures
30 °C, 40 °C and 50 °C.

Samples bamboo fiber 200mg	Temperature ° C	**CI (%)**	**Crystalline (%)**	**Crystallite size (nm)**	**Z-value**
Control	30	48	65	2.31	-21.9
Treated		49	66	2.97	-24.5
Control	40	41	63	2.82	-18.0
Treated		57	72	3.91	-34.6
Control	50	51	67	2.62	-29.7
Treated		55	69	3.11	-34.1

The lignin and other impurities (hemicellulose, pectin, wax) in the bamboo fibers dissolve gradually and get completely removed at the temperature 40 °C, resulting in the hyper increasing of the crystallinity index.
Another parameters are tabulated in table (1) which estimated the content of crystalline (%) and the crystallite size of treated bamboo fiber that observed higher in the temperature 40 °C as calculated to be 72%, 3.91 nm compared to the temperature 30 °C and 50 °C as reported a 66%, 2.97nm and 69%, 3.11nm respectively. This indicates that the crystallinity index raised with rising the content of crystalline (%) and the crystallite size which are related to the removal of the amorphous domains.
Finally, there really are two types of cellulose natures which include I_α and I_β cellulose structure. The I_β structure was observed only in this method since it only appears in higher plants, while I_α diminished due to the presence only in bacterial and alga celluloses [11].

The crystalline cellulose I_α and I_β structures are belong to monoclinic and triclinic unit cells, respectively [12]. As illustrated in table (1), the Z values of bamboo fibers investigated that the cellulose structure was of the I_β dominant type.

Conclusions

This study investigated the crystallinity characterization of untreated and treated bamboo fibers using X-ray diffraction analysis. Compared to untreated bamboo fibers, a smoother surface, and more surface area showed through removal of amorphous non-cellulosic compounds i.e lignin, hemicellulose, pectin in the treated bamboo fibers in all temperatures 30°C, 40°C and 50°C but especially in 40°C estimated completely removal of cellulosic impurities altered the nature of fiber to extra crystalline that is indicated from the XRD analysis.

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