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Enzymatic hydrolysis and characterization of lignocellulosic biomass exposed to electron beam irradiation

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ABSTRACT

Pretreatment of lignocellulosic biomass has been taken up as a global challenge as it comprises a large renewable source of fermentable sugars. In this study, effect of electron beam irradiation (EBI) on a hybrid grass variety investigated as a biomass pretreatment method. Dry biomass samples after characterization were exposed to EBI doses of 0, 75, 150 and 250 kGy. The pretreated biomass samples were enzymatically hydrolyzed using *Trichoderma reesei* ATCC 26921 cellulase for 144 h. The enzyme loadings were 15 and 30 FPU/g of biomass. The structural changes and degree of crystallinity of the pretreated biomass were studied by FTIR, XRD and SEM analyses. The lignocellulosic biomass sample showed 12.0% extractives, 36.9% cellulose, 28.4% hemicellulose, 11.9% lignin and 8.6% ash. Significant improvements in the reducing sugar and glucose yields were observed in the hydrolysate of EBI pretreated biomass compared to the control. In 250 kGy exposed samples 79% of the final reducing sugar yield was released within 48 h of hydrolysis at an enzyme loading rate of 30 FPU/g of biomass. The IR crystallinity index calculated from the FTIR data and degree of crystallinity (XRD) decreased in the EBI treated samples. A significant negative correlation was observed between degree of crystallinity and the glucose yield from enzymatic hydrolysis.

1. Introduction

Pretreatment of lignocellulosic biomass has become an unavoidable step while processing the biomass for the renewable production of bio-based materials such as fuels, chemicals and energy. Lignocelluloses are commonly available in abundance as agricultural and forest residues. The plant derived biomass is complex due to the presence of intricate intermolecular arrangements of lignin, hemicellulose and cellulose and limits its direct application in the production of value added goods (Himmel et al., 2007). The polymeric structure of cellulose also makes it recalcitrant for the enzymatic conversion into sugars and demands an intermediary step for the production of ethanol and other biomaterials (Zakzeski, Bruijnincx, Jongerius, & Weckhuysen, 2010).

Pretreatment methods aim at facilitating maximum saccharification of cellulose by enzymatic hydrolysis. It is found that cellulose from untreated lignocellulosic biomass upon enzymatic hydrolysis can yield not more than 15–25% glucose due to the recalcitrance (Zheng, Pan, & Zhang, 2009). This has led to intense research on developing feasible pretreatment methods and significant improvements in the biomass pretreatment technology have been made during the last decade (Hendriks & Zeeman, 2009; Lloyd & Wyman, 2005; Shen et al., 2011).

Most of the pretreatment methods have some disadvantages in terms of cost, recovery, secondary pollution, and formation of intermediary compounds that inhibit enzymatic hydrolysis (Carter, Squillace, Gilcrease, & Menkhaus, 2011; Zheng et al., 2009). Advanced technologies such as laser, microwave and electron beam irradiation have become the recent research attraction due to its fast and effective results and have been experimentally used to study the changes caused to the biomass (Binod et al., 2012; Tian, Wang, Fan, & Zuo, 2011). Other important pretreatment methods currently under investigation include the use of ionic liquids (Binder & Ronald, 2010) and super critical carbon dioxide pretreatment (Santos, Kawase, & Coelho, 2011).

Advances in nuclear physics have led to the creation of various facilities to render the goods of radiation for improving the quality of life. And the use of associated technology has also been experimentally extended to improve the saccharification of cellulosic biomass (Khan, Ahmad, & Kronfli, 2006; Yang, Shen, Yu, & Wang, 2008). Biomass pretreatment with EBI at high doses effectively enhances the enzymatic saccharification (Bak et al., 2009). Major changes that EBI bring about in the cellulose are, decrease in the crystallinity and molecular weight and increase in the surface area (Driscoll et al., 2009). Preliminary research studies have evidenced

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Table 1
Comparative account on some of the previous studies that reported increased hydrolysis of biomass after EBI exposure highlighting variation in the effective EBI doses and enzyme system used.

Sl. No.	Biomass samples	EBI dose	Enzyme system used	Glucose yield (%)		References
				Control	EBI treated	
1	Rice straw (finely milled)	80 kGy	60 FPU cellulase (Celluclast 1.5 L) + 30 CBU β-glucosidase (Novozyme 188)	35	52	Bak et al. (2009)
2	Industrial hemp (finely milled)	450 kGy	20 FPU/g biomass Celluclast 1.5 L supplemented with Novozym 342	29	35	Shin and Sung (2008)
3	(i) Corn stover (ii) Peanut husk (finely milled)	500 kGy	0.5 g of cellulase per g biomass (Onozuka R-10)	20 4.5	43 20	Chosdu, Hilmy, Erizal, Erlinda, and Abbas (1993)
4	Rice straw (milled) treated with 4% NaOH	50 Mrad (50 kGy)	0.5% cellulase per g biomass (Onozuka R-10)	12	36	Xin and Kumakura (1993)
5	Newsprint	1 MGy	30–35 FPU/g of material (*Trichoderma reesei* RUT C-30)	5	90[a]	Khan et al. (1987)
6	Pulp and paper mill sludge	1.5–2 MGy	-do-	–	90[a]	-do-

[a] Values indicate saccharification rate as calculated by reducing sugar content and cellulose.

improvements in the enzymatic saccharification of cellulosic material after EBI treatment. It is also important to emphasize that the effectiveness of the EBI pretreatment on the rate of enzymatic hydrolysis depends on the nature of the biomass with respect to energy delivered, sources and concentration of enzymes used. Earlier research studies have shown difference in the hydrolysis rate for different biomass samples used and are summarized in Table 1. Due to the efficiency of industrial scale EBI machines, a high dose rate can be achieved at shorter time period with good utilization of energy and development of indigenous industrial scale EBI facilities has opened up opportunities for researchers.

India is a populous fast developing nation with rapid industrialization has a high demand for alternative energy. Lignocellulose based alternative energy provides a promising solution for the efficient management of natural resources, reducing the contributions to climate change and maintaining environmental sustainability. Though, India produces ethanol from molasses, it may not be sufficient to sustain the increasing demands for fuel. Hence, in this study a fast growing perennial hybrid grass variety was used for studying the effect of EBI on the enzymatic saccharification and structural changes in the lignocellulosic composition.

2. Materials and methods

2.1. Biomass, sample preparation and compositional analysis

A hybrid grass variety developed in India by crossing hybrid napier grass (*Pennisetum typhoides* × *P. purpureum*) with bajra (*P. glaucum* L.) was used as the lignocellulosic biomass. The above ground biomass was collected from the farms around Mangalore region (India). Freshly cut biomass (85% moisture) was washed, dried completely and powdered using a laboratory analytical mill (IKA, Germany). The milled samples were sieved (2 mm), packed in air tight bags and stored at room temperature until EBI treatment. The biochemical composition of the biomass was analyzed following the standard methods. The extractives were determined for the biomass samples by extracting a known weight of the biomass with 80% ethanol at 80 °C using soxhlet apparatus for 6 h. Ash and lignin were analyzed using Biomass Analytical Procedures of National Renewable Energy Laboratory, USA (NREL, 2008). Cellulose content in the biomass was measured by Updegraff method using avicel cellulose (Sigma, USA) as standard (Updegraff, 1969). Hemicellulose content was calculated as the difference between acid detergent fiber and neutral detergent fiber contents (Goering & Van Soest, 1973).

2.2. Pretreatment by electron beam irradiation

The EBI exposure studies were conducted in the Electron Beam Centre (EBC, BARC), Kharghar Mumbai (India). The industrial scale EBI facility developed for material processing has a linear accelerator of 10 MeV (10 kW RF Linac, BARC, India) and employs the 100 mA, 10 μs current pulses repeating at 300 Hz to scan the samples (Mittal et al., 2005). For EBI exposure, milled and sieved biomass samples (150 g) were packed in airtight polythene bags (180 mm × 120 mm × 20 mm). The doses selected were 75 kGy (G75), 150 kGy (G150) and 250 kGy (G250). Samples were uniformly exposed to EBI by 10 mm diameter beam scanning over 1 m length in a conveyor. The conveyor speed was fixed at 0.1 m/min to deliver a dose of 21 ± 2 kGy per pass. The selected doses were achieved by calculating the number of passes required. Simultaneously, the dose accumulated was confirmed by radio chromatic films. The untreated biomass (GC) was used as control.

2.3. Enzymatic hydrolysis

The enzymatic saccharification was carried out using extractive free EBI treated and control biomass samples. For the enzymatic saccharification cellulase from *Trichoderma reesei* ATCC 26921 (Sigma, USA) was used at the rate of 15 and 30 FPU/g of biomass. Enzymatic hydrolysis of pretreated samples (1 g each) was carried out in 50 mM citrate buffer (pH 4.8) at 10% (w/v) substrate consistency (NREL, 2008). The substrate with buffer was pre-incubated at 50 °C prior to the addition of enzymes. Tetracycline (400 μg) and cyclohexamide (300 μg) were used to control the growth of microorganisms. At regular intervals, a small amount of hydrolysate was cultured on nutrient agar (Himedia, India) and potato dextrose agar (Himedia, India) plates to ensure that the hydrolysis mixture was free from contamination by bacteria and fungi, respectively. At the end of the hydrolysis (144 h) the samples were filtered, residue was collected and oven dried (55 ± 5 °C) for further analysis. Progression of enzymatic hydrolysis was monitored at regular intervals (24, 48, 72, 96, 120 and 144 h) by estimating the reducing sugar in the hydrolysate using dinitrosalicylic acid method (Miller, 1959) and the glucose content by glucose oxidase method (Werner, Rwy, & Wielinger, 1970). The theoretical glucose yield was calculated as follows:

$$\text{Glucose yield (Theoretical)}\,\% = \left\{\frac{\text{g of glucose released}}{(\text{g glucan} \times 1.1)}\right\} \times 100$$

2.4. Fourier transform infrared (FTIR) spectroscopy

The structural changes in the lignocellulosic biomass with respect to EBI treatment and enzymatic hydrolysis were analyzed by FTIR spectroscopy. For this, biomass sample (10 mg) was pelleted by mixing with spectroscopic grade KBr and FTIR spectrum was recorded between 4000 and 400 cm^{-1} using Shimadzu spectrometer (Prestige 21, Shimadzu, Japan) at 1 cm^{-1} resolution and 10 scans per sample. The IR crystallinity index of cellulose was calculated as the intensity ratio between IR absorptions at 1427 and 895 cm^{-1} which are assigned to CH_2 bending mode and deformation of anomeric CH, respectively (Åkerholm, Hinterstoisser, & Salmén, 2004; Kataoka & Kondo, 1998).

2.5. XRD analysis

The biomass samples (10 mg each) were examined by XRD using powder X-ray diffractometer with Si (Li) PSD detector (Bruker AXS D8 Advance, Germany). The operation voltage and current were maintained at 40 kV and 35 mA, respectively, and an angular range 3–135°. The diffraction spectra were collected using θ–2θ method. The samples were scanned from 2θ range of 3–80° in steps of 0.020°. Radiation (Cu Kα X-ray source) wavelength was 1.5406 Å. The crystallinity index (CrI) was calculated from the intensities of crystalline region between 2θ = 21.9–22.1° and the amorphous region at 2θ = 18° by the following equation (Segal, Creely, Martin, & Conrad, 1959):

$$\text{Crystallinity index (CrI)} = \left\{\frac{(I_{cr} - I_{am})}{I_{cr}}\right\} \times 100$$

where I_{cr} is intensity in the major peak appropriately at 2θ = 21.9–22.1° and I_{am} is the intensity at 2θ = 18°.

The degree of crystallinity was calculated as the relation between the area under crystalline peaks and non-crystalline region using following equation:

$$X_c = \left\{\frac{F_c}{(F_a + F_c)}\right\} \times 100$$

where X_c is degree of crystallinity, F_c and F_a, respectively, are the area of crystalline and non-crystalline regions.

2.6. Scanning electron microscopy (SEM)

For the SEM analysis, biomass samples were prepared onto adhesive carbon tape on an aluminum stub followed by gold sputter coating. Surface morphology of the sample was studied using analytical scanning electron microscope (JSM-6380LA, JEOL, USA).

2.7. Statistical analysis

Mean and standard deviation values of data were calculated from four repeats. The data on the enzymatic hydrolysis was analyzed for significance in the differences between treatments using one-way analysis of variance (ANOVA). Correlation between the degree of crystallinity and glucose yield was tested by correlation regression analysis. All the statistical analyses were performed using a software package, STATISTICA (Statosoft, USA, 1998). Differences were declared as significant at p value < 0.01 unless specified.

3. Results

3.1. Biomass composition

The compositional analysis of the EBI treated samples with respect to control revealed that the total extractives and cellulose contents did not vary significantly between treatments. The total extractives were 12.0%, 11.0%, 11.2% and 11.7% for GC, G75, G150 and G250, respectively. The cellulose content was 36.9%, 35.2%, 34.8% and 34.0%, respectively, for GC, G75, G150 and G250. The hemicellulose content in G250 samples (11.7%) was significantly lower than the control (28.4%), G75 (27.5%) and G150 (24.5%). Ash and lignin contents in the control biomass, respectively, were 8.6% and 11.9%.

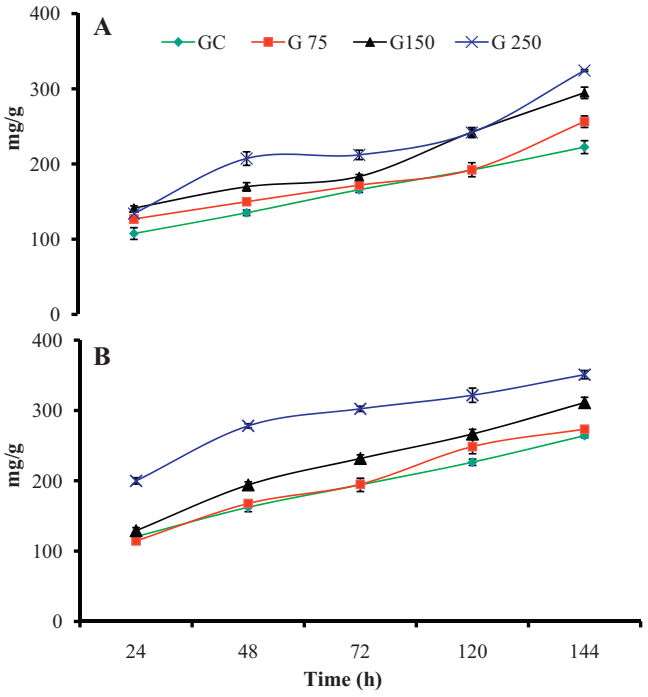

Fig. 1. Temporal variation in the reducing sugar yield during hydrolysis of the biomass samples exposed to 0, 75, 150 and 250 kGy of EBI, using *T. reesei* cellulase (Sigma) at enzyme loadings of (A) 15 FPU/g of biomass (B) 30 FPU/g of biomass. Data points are mean ± SD of four repeats.

3.2. Effect of EBI on enzymatic hydrolysis

The reducing sugar released during the enzymatic hydrolysis from the biomass samples exposed to different doses of EBI is given in Fig. 1. Significant improvement in the enzymatic saccharification of the EBI exposed biomass was observed compared to control. By the end of 144 h of hydrolysis, compared to all the other treatments, significantly higher reducing sugar yield was recorded for the G250 treatment at both low (15 FPU/g of biomass) and high (30 FPU/g of biomass) enzyme loadings. The reducing sugar yield was 8.23% higher at 30 FPU/g of biomass than 15 FPU/g of biomass enzyme loading for G250 treatment. In the same treatment, with an enzyme loading of 15 FPU/g of biomass, the reducing sugar yield was 46%, 27% and 10% higher than GC, G75 and G150 treatments, respectively, and almost similar increase was also observed with 30 FPU/g of biomass enzyme loading. The biomass treated with 75 kGy and 150 kGy of EBI, the reducing sugar concentration increased, respectively, by 15% and 32% compared to control at 15 FPU/g of biomass.

It is interesting to note that more than 55% of the final (144 h) reducing sugar content was released within 48 h of incubation in all the treatments including control irrespective of the final yield. However, in G250 treatment, 79% of the final reducing sugar content was released within 48 h at 30 FPU/g of biomass. Significant increase in the glucose yield compared to control in the EBI exposed samples was also observed (Fig. 2). The theoretical glucose yield using 15 FPU/g of biomass was 37% for the G250 sample and that was 17% higher than the control.

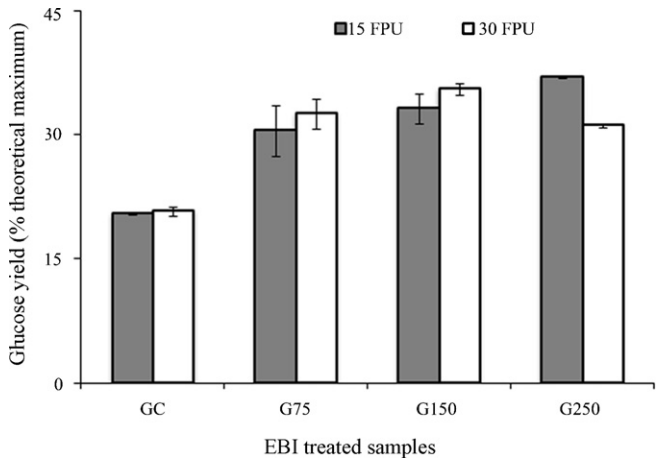

Fig. 2. Theoretical glucose yields in control and EBI treated biomass samples after 144 h of hydrolysis using *T. reesei* cellulase (Sigma) at enzyme loadings of 15 and 30 FPU/g of biomass. Data points are mean ± SD of four repeats.

3.3. Effect of EBI on structure and crystallinity of the biomass

3.3.1. FTIR analysis

The FTIR spectra of EBI treated, hydrolyzed and control biomass samples are given in Fig. 3 (a–c). The IR band in the region 898 cm^{-1} corresponding to β anomers or β-linked glucose polymer (Wang, Wang, & Feng, 2010) showed slight shifts (895 cm^{-1}) in the EBI treated samples (G150 and G250) (Fig. 3a). Decreased intensities in this region were observed in the IR spectra of the EBI treated biomass residue obtained after the hydrolysis (15 and 30 FPU) (Fig. 3b and c). Similarly, IR bands at 1373 cm^{-1} characteristic of C—H deformation (symmetric) of cellulose (Theerarattananoon et al., 2010) showed a slight shift toward 1375 cm^{-1} (G75) and 1377 cm^{-1} (G150 and G250) and the intensity of this peak was very low for the hydrolyzed residual biomass samples particularly G150 and G250 treatments. Differences in the intensities among control, EBI treated and hydrolyzed samples were also observed at 1427 cm^{-1} IR region corresponding to C=C stretching of the aromatic ring in the biomass (Wang et al., 2010). The IR bands at 1000–1200 cm^{-1} region of the spectra are attributed to structural features of cellulose and hemicellulose (Marchessault & Liang, 1960). Formation of new shoulder peaks in this region was observed in the spectra obtained for the hydrolyzed biomass residue of the EBI treated samples (Fig. 3b and c) with overall decrease in the intensity in the region. Other major changes observed in the IR spectra are summarized in Table 2.

3.3.2. XRD of EBI exposed biomass

The XRD analysis revealed characteristic pattern for the biomass with a predominant cellulosic peak (0 0 2 plane) at $2\theta = 22.1^\circ$, 22.0°, 22.1° and 21.9°, respectively, for the control, G75, G150 and G250 samples (Fig. 4). The intensity of the major peak decreased with increasing dose of EBI indicating reduced crystallite size or degree of crystallinity.

3.3.3. IR crystallinity index and degree of crystallinity

IR crystallinity index, degree of crystallinity and crystallinity index calculated from FTIR and XRD are given in Table 3. From the FTIR spectral data, IR crystallinity ratio was calculated for the EBI treated and control biomass samples based on the absorption at characteristic IR bands at 1427 and 895 cm^{-1}. A decrease in the IR crystallinity A_{1427}/A_{895} ratio was observed for EBI treated samples indicating changes in the crystallinity of cellulose in the biomass. The IR crystallinity index obtained for the residual biomass samples after the hydrolysis (144 h) using 15 FPU/g biomass enzyme loading showed comparatively higher values of 0.79, 0.69, 0.62 and 0.65, respectively, for GC, G75, G150 and G250. But, the residual samples obtained after hydrolysis with 30 FPU/g biomass enzyme loading showed lower indices of 0.57, 0.60, 0.56 and 0.68 for GC, G75, G150 and G250, respectively.

XRD crystallinity index showed an increase from control to treated biomass. However, the degree of crystallinity decreased from control to G250 in the order of increasing EBI dose. A maximum decrease of 29% was observed between control biomass sample and samples exposed to 250 kGy.

3.4. SEM analysis

The SEM of the biomass samples showed changes in the biomass morphology particularly after the EBI treatment and the residual biomass obtained after the hydrolysis at 15 and 30 FPU/g biomass exposing the degree of damages in the crystalline cellulose fibers (Fig. 5a–c). Many localized damages were observed in the micrographs of EBI treated samples and in the hydrolyzed biomass residue.

4. Discussion

The global demand for developing an ideal fuel crop in a sustainable way forces scientists and farmers to redesign agricultural approaches for the improved crop and its production (Stiklen, 2008). Indigenously developed hybrid grass variety suitable for growing in the tropical climate was used in this study as a biomass feedstock. This hybrid grass showed promising features as a suitable fuel crop in terms of the structural composition and availability.

The enzymatic hydrolysis was favorably affected by EBI pretreatment of biomass which may be due to the structural changes in the lignocellulosic polymer network that has been caused by the high energy irradiation. Similar observations were documented earlier for other cellulosic materials. Newsprint and paper mill wastes required doses as high as 1.5–2 MGy to achieve 90% saccharification by *T. reesei* cellulase (Khan, Labrie, & McKeown, 1987). Enzymatic saccharification of corn stalk biomass treated with 2% NaOH and exposed to EBI (500 kGy) yielded 20% more glucose compared to the control within 48 h of hydrolysis (Chosdu et al., 1993) and similar increase (30%) in glucose yield compared to control was recorded for the 80 kGy (EBI) treated rice straw samples after 132 h of enzymatic hydrolysis (Bak et al., 2009). In the present study, 46% increase was observed in the reducing sugar yield at 15 FPU/g of biomass for the 250 kGy exposed samples compared to control. The theoretical glucose yield in 250 kGy exposed samples at 48 h of hydrolysis (15 FPU/g of biomass) was 8% more than the control and continuing the hydrolysis further to 144 h yielded 17% more glucose. It should be noted that the hydrolysis was carried out using *T. reesei* cellulase, and improvements could have been expected if, more efficient enzyme mixtures were used.

Due to the meager research on EBI pretreatment for biomass hydrolysis, the published data show variations in the results and comparisons of rate of glucose yield are not consistent with dose of EBI (Bak et al., 2009; Shin & Sung, 2008). The variations in the results may primarily be due to the different types of biomass samples used, differences in the lignocellulosic compositions, enzyme preparations used and the loading rates. Hydrolysis of pretreated cellulosic materials can be improved further by using efficient cellulase enzyme systems (Abdel-Fattah & Abdel-Naby, 2012; Zhang & Lynd, 2004).

Enzymatic hydrolysis of cellulose has widely been accepted to be dependent on the crystallinity and pretreatments play an important role in decreasing the crystallinity (Cheng et al., 2011; Park,

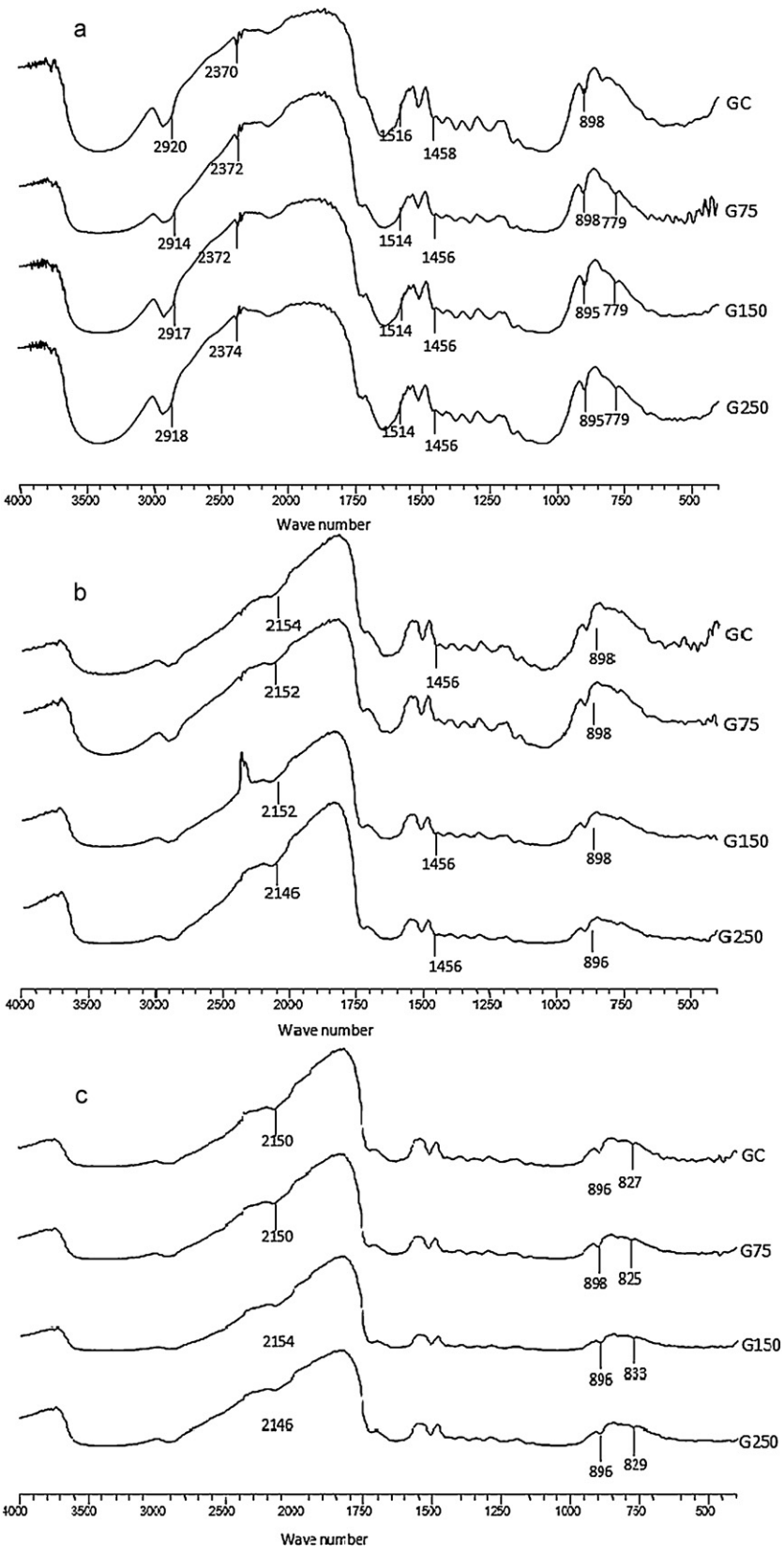

Fig. 3. FTIR spectra of (a) control and EBI treated biomass samples. (b) Control and EBI treated biomass residue obtained after 144 h hydrolysis using 15 FPU/g biomass. (c) Control and EBI treated biomass residue obtained after 144 h hydrolysis using 30 FPU/g biomass.

Baker, Himmel, Parilla, & Johnson, 2010). Significant increase in hydrolysis rate for the EBI treated biomass can be correlated to the decreased crystallinity caused by high energy EBI and in this study, a significant ($p < 0.05$) negative correlation ($r^2 = 0.95$) was observed between glucose yield (15 FPU/g of biomass) and degree of crystallinity. Decrease in the degree of crystallinity (XRD) is also an indicator of decrease in the degree of polymerization and increase in the surface area which in turn increase the hydrolysis of

Table 2
Characteristic IR bands present in the lignocellulosic biomass and the changes observed for the (a) EBI treated and (b) for biomass residue obtained after hydrolysis at 15 FPU/g biomass and (c) at 30 FPU/g biomass (GC-control, G75, G150 and G250, respectively, are biomass samples exposed to 75, 150 and 250 kGy of EBI). The characteristic IR bands for cellulosic materials are summarized from Kataoka and Kondo (1998); Marchessault and Liang (1960); Nelson and O'Connor (1964); Wang et al. (2010).

Samples		Characteristic IR bands					
		829 cm^{-1} α anomers in side chains	1161 cm^{-1} Arabinosyl side chains or C—O—C stretching for glucose ring	1458 cm^{-1} Aromatic C—H vibrations	1638 cm^{-1} Bending of absorbed residual water	1733 cm^{-1} Hemicellulosic sub-fractions	2922 cm^{-1} C—H stretching vibrations
GC	a	829	1161	1458	1638	1720	2922
	b	827	1157	1456	1635, 1651	1733	2926
	c	827	1159	1458	1633	1726	2920
G75	a	779	1159	1458	1639	1720	2900
	b	825	1161	–	1629, 1654	1716, 1726	2922
	c	825	1161	1456	1627	1726	2914
G150	a	779	1159	1460	1641	1722,1732	2922
	b	831	1159	1456	1631	1726	2918
	c	833	1159	1456	1631	1726	2917
G250	a	779	1161	1460	1641	1722, 1732	2922
	b	830	–	1456	1631	1724	2920
	c	829	1161	1456	1631	1724	2918

Table 3
IR crystallinity ratio calculated from FTIR intensities, degree of crystallinity and crystallinity index measured by XRD for the control and EBI treated biomass samples (GC-control, G75, G150 and G250, respectively, are biomass samples exposed to 75, 150 and 250 kGy of EBI).

Samples	IR crystallinity A_{1427}/A_{895}	Degree of crystallinity (%)	Crystallinity index (%)
GC	0.61	50	67
G75	0.55	35	67
G150	0.44	23	87
G250	0.58	21	87

lignocellulosic materials depending upon the biomass sample used (Driscoll et al., 2009; Hendriks & Zeeman, 2009). Variation in crystallite size affects the observed diffraction peak and such variations have been observed after the pretreatment of the biomass (Cheng et al., 2011). Whereas, increase in the crystallinity index (XRD) may be due to decrease in the amorphous region in relation to the crystalline peak for the EBI exposed biomass. It is known that, when the amorphous domains of the cellulose are attacked, chain scission occurs reducing the amorphous cellulose and thereby increasing the crystallinity index (Gumuskaya, Usta, & Kirci, 2003). This is also supported by the increased IR crystallinity indices for the residual biomass samples obtained after hydrolysis using lower enzyme loading (15 FPU/g biomass) indicating the possible presence of highly crystalline cellulose that were recalcitrant for enzymatic attack. But, doubling the enzyme loading (30 FPU/g biomass), however, decreased indices except for G250 compared to the lower enzyme loaded biomass residues. The differences between the EBI treated and control biomass clearly indicate that the decreased crystallinity was the major favorable effect of EBI on the hydrolysis of biomass.

The acid, alkali and other chemical treatments aid in the removal of lignin and hemicellulosic components from the biomass, but, result in the formation of inhibitory compounds such as furfural, hydroxymethyl furfural (HMF) and acetic acid (Kumar, Barrett, Delwiche, & Stroeve, 2009). The inhibitory compounds significantly reduce the hydrolytic efficiency and needs extended steps for their removal which is inevitable for the efficient saccharification process (Zheng et al., 2009). Hence, physical methods can be considered as better options for the pretreatment of biomass over the chemical methods. One of the important features of the EBI pretreatment is the dissociation of the lignocellulosic complex without forming inhibitory compounds (Bak et al., 2009). This may be due to the cleavage of chemical links through rapid localization of the absorbed energy within the molecules leading to the formation of free radicals (Onyenekwe, Stahll, & Greiner, 2010).

Decrease in IR crystallinity index of cellulose by indicates changes occurred in the cellulose molecule due to EBI treatment. Morphological changes observed from SEM and changes in the characteristic IR bands for lignin, hemicellulose and cellulose in the treated samples contribute to the understanding

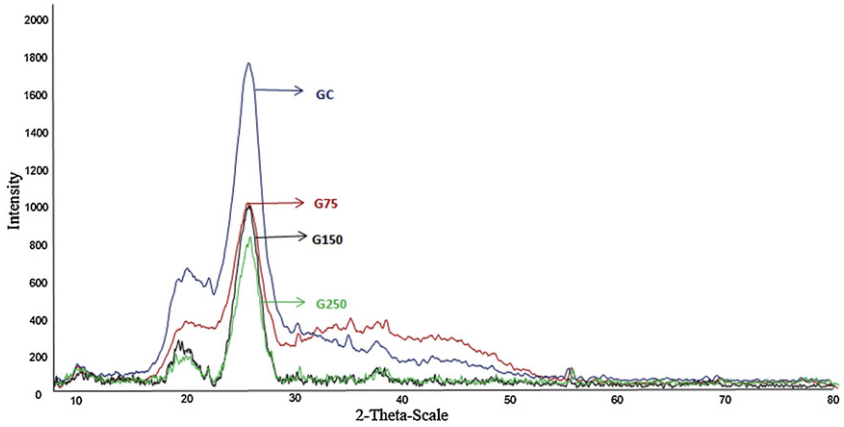

Fig. 4. X-ray diffractogram of control and EBI treated biomass samples.

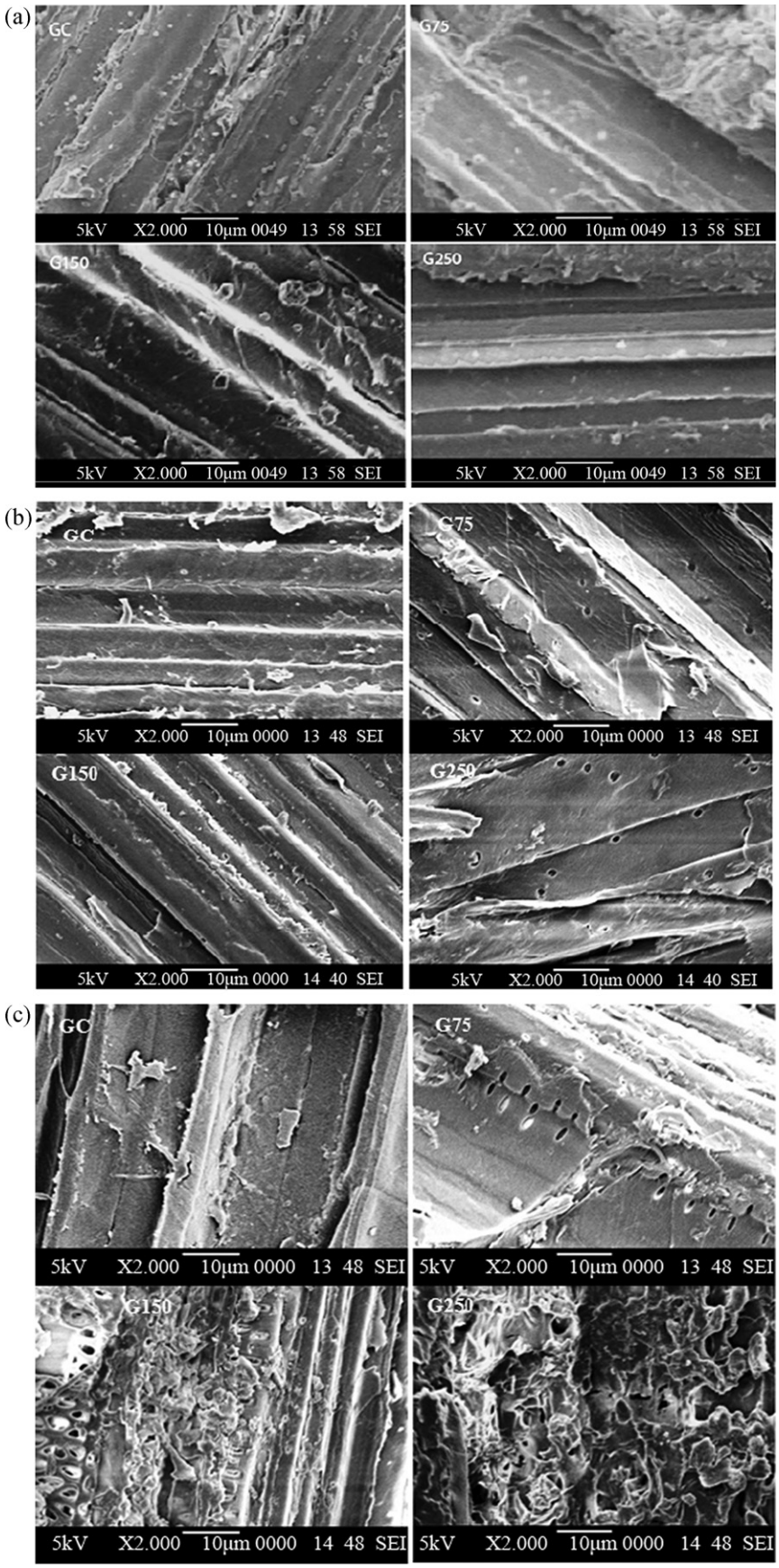

Fig. 5. Representative SEM images of (a) control and EBI treated biomass samples. (b) Control and EBI treated biomass residue obtained after 144 h hydrolysis using 15 FPU/g biomass. (c) Control and EBI treated biomass residue obtained after 144 h hydrolysis using 30 FPU/g biomass.

that, EBI can bring about structural changes in the macromolecular lignin–hemicellulose–cellulose complex. It is indeed difficult to investigate all the changes caused by the EBI on the cellulose molecule that have contributed to significant improvements in the enzymatic saccharification of the biomass.

Any effective pretreatment method should be fast reliable, sustainable and environmentally friendly. A vast scope exists for utilizing EBI or modifications thereof for employing as an effective pretreatment method for the lignocellulolsic biomass. Though concerns over the cost and effectiveness of the EBI pretreatment persist, other factors such as environmental sustainability and future demands for fuel and chemicals shall not limit the research efforts on utilizing EBI considering the vast array of value added goods that can be generated by effective pretreatment of cellulosic materials.

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