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The effects of electron beam on fiber morphology and ruminal fiber degradation characteristics in sugarcane bagasse

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Abstract

The purpose of this study was to evaluate the effects of electron beam irradiation on fiber morphology and ruminal fiber degradability of sugarcane bagasse. Samples of untreated or electron beam irradiated sugarcane bagasse were subjected to the rumen of three fistulated rams and ruminal degradation parameters of neutral detergent fiber were determined. The association of rumen bacteria with tissues of the sugarcane bagasse during ruminal degradation was investigated by scanning electron microscopy. Based on the results, electron beam irradiation increased the water soluble fraction, degradable fraction, degradation rate and ruminal effective degradability of neutral detergent fiber as dosage increased ($P < 0.05$). In untreated bagasse, there was a natural structure of fiber in the surface with large particle size, multiple layers, small pores and cavities. Irradiation at doses of 250 and 500 kGy resulted in the breakdown of the fiber, drastically reduction in particle size of the fibers and smooth edge. The electron beam irradiation increase or deepen the cleavages. Scanning electron microscopy images showed that at dose of 500 kGy, there was a higher activity of fibrolytic bacteria on the surface of fibers. In this irradiation dose, the penetration of microorganisms and also layer separation was higher than dose of 250 kGy. In addition small particles were not observed on the surface, but irregular fractures and folds observed. The data suggest that electron beam irradiation at dose of 500 kGy could change fiber morphology and improve ruminal fiber degradability of sugarcane bagasse.

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Introduction

Bagasse, the residue after sugar removal from sugarcane, is a lignified material in the sugar industry. In Khozestan province of Iran, sugarcane is cultured in the wide extension and the treated form of this material is a biomass source that can be fed to feedlot cattle. Some physical and chemical treatments have been applied to cut the linkage between lignin and cellulose and to enhance its nutritional value (Basile, and Machado, 1990; Deschamps *et al.,* 1996). Although these methods have many benefits, but producing furfurans in physical processing that is poisonous substances for ruminal microbes and the hazards of chemical materials for animal resulted in limit of their application. Electron beam irradiation has been used as an enhancing method for the enzymatic hydrolysis (Takács *et al.,* 1999; Kuczumow *et al.,* 1999) in cellulose industry, and thus improving in digestibility of crop residue (Al-Masri and Zarkawi, 1994). In the literature, effects of electron beam irradiation on fiber digestibility (Yang *et al.,* 2008; Shawrang *et al.,* 2011, 2013) and cellulose properties (Kumakura M and Kaetsu, 1978; Alberti *et al.,* 2005; Bouchard *et al.,* 2006; Driscoll *et al.,* 2009) were evaluated, but there was limited information about the effects of electron beam irradiation on the microscopic and fiber structure of roughages. Therefore, the objective of our study was to characterize the response of sugarcane bagasse to electron beam irradiation, in terms of fiber morphology and degradability.

Materials and methods

Sample preparation and irradiation treatments

Bagasse sample was collected from Haftape sugar refinery in Khuzestan province of Iran and dried at 55 °C for 5 days. After hand mixing, bagasse was divided to three batches to form triplicate sources for irradiation processing. Bagasse sample were packed in nylon bags and then were exposed to electron beam irradiation (Rhodotron accelerator model TT200, IBA co., Belgium) at the Yazd radiation processing center (AEOI, Yazd center, Iran) to doses of 250 and 500 kGy. Multiple passages (9 pass for 250 kGy and 18 pass for 500 kGy) were used to obtain the needed doses.

Ruminal degradability

Three castrated Shal rams with an average weight of 65 kg with rumen fistulas were used for *in sacco* trial**.** Nylon bags with a pore size of 45 µm were packed with three grams of the samples milled to pass a 2 mm screen based on the standard method of Ørskov and McDonald (1979). Triplicate bags packed with untreated or irradiated bagasse were inserted in the rumen for periods of 0, 6, 12, 24, 48, 72 and 96 h. Two series of incubations were conducted for each bagasse batches. After removal from the rumen, nylon bags were washed with water and stored at -20 °C. After thawing, nylon bags were washed two times for 8 min in a washing machine. The same method was conducted to two series of two bags to obtain the zero hour value. The residues were dried and then analyzed for dry matter (DM) and neutral detergent fiber (NDF) to establish degradation kinetics of bagasse.

Chemical analyses

Moisture content was determined before and after samples were stored overnight in an oven at 105 °C (AOAC, 1995). Nitrogen was determined using a Dosimat-776 Metrohm apparatus (Metrohm Co., Switzerland) (AOAC, 1995). Fat content was determined with a Solvent Extractor (Behr Labor-Technik, Düsseldorf, Germany) equipped with six Soxhlet posts. The ether extract was determined according to the method of AOAC, 1995. Ash was determined by burning triplicate 1 g samples at 540 °C, for 3 hour in a muffle furnace (AOAC, 1995). Neutral detergent fiber was analyzed according to the method of van Soest *et al.* (1991), using an automatic fiber analyzer (Velp Scientifica, Milan, Italy).

Scanning electron microscopy (SEM)

The surface characteristics and microbial attachment status of sugarcane fiber were all observed by scanning electron microscopy (SEM), (S4700, HITACHI). The acceleration voltage was 10 kV. The samples were coated with Os using a vacuum sputter coater.

Statistical analyses

Disappearances of fiber in the rumen were fitted for each ram to the exponential model of Ørskov and McDonald (1979). Data were analyzed using the general linear models procedure of SAS (1996) based on completely randomized design. Differences among treatments were separated using Duncan Multiple testes (Steel and Torrie, 1980).

Results and discussion

Effects on fiber degradability

Fiber degradation kinetic of untreated and irradiated sugarcane bagasse is reported in Table 1. The soluble fraction (a), degradable fraction (b), degradation rate (c) and effective degradability of fiber increased as irradiation dose increased ($P < 0.05$). Similar degradability of fiber was reported by Gralak *et al.* (1994) in roughages. Also, Leonhardt *et al.* (1983) showed that fiber digestibility of some straws can increase by treatment with gamma rays or accelerated electron. Under beam irradiation, cell wall constituents undergo degradation which is due to the breaking off the glucosidal bond and modification in their structures (Takács *et al.* 1999). Modification in fiber structure may be due to several factors that lead to the opening of the anhydroglucose ring. In addition, The improvement in digestibility of fiber with irradiation observed is likely to be due to a combination of the decreased particle size, increasing the surface area exposed for microbial attachment, a possible increase in solubility, the alteration in the chemical composition, and decomposition of cellulose and hemicellulose and seriously weakens the cellulosic fiber (Yang *et al.*, 2008; Driscoll *et al.,* 2009). Moreover, reducing in crystallinity of cellulose (Iller *et al.,* 2002; Kasprzyk *et al.,* 2004) is a reason of increasing digestibility of irradiated sugarcane bagasse. Also, the link of lignin with other compounds in cell wall is broken by irradiation (Wasikiewicz *et al.,* 2005).

Table 1. Fiber degradation parameters of untreated and irradiated sugarcane bagasse [a].

	Degradation traits [b]			ERD (g/kg) at outflow rate (/h)		
	a (g/kg)	b (g/kg)	c (%/h)	0.02	0.05	0.08
Untreated	59[c]	313[c]	1.9[c]	369[c]	364[c]	359[c]
250 kGy	71[b]	357[b]	2.2[b]	425[b]	420[b]	415[b]
500 kGy	90[a]	430[a]	2.6[a]	517[a]	512[a]	507[a]
SEM	16.8	23.6	0.28	14.0	19.7	23.3

[a,b,c] Means in the same column with different superscripts differ ($P < 0.05$).

Effects on fiber morphology

The scanning electron microscopy (SEM) observation of untreated bagasse and irradiated at doses of 250 and 500 kGy are shown in Figure 1. The 800-times magnified images of control and processed bagasse provided a clue of how processes physically cut and changed fibers. In Fig.1a (untreated bagasse), there is a natural structure of fiber in the surface with large particle size, multiple layers, small pores and cavities and relatively fuzzy edges. Irradiation at doses of 250 (Fig. 1b) and 500 kGy (Fig. 1 c) resulted in the breakdown of the fiber, drastically reduction in particle size of the fibers and smooth edge. The electron beam irradiation increase or deepen the cleavages. In irradiated samples, the edge of bagasse fiber pieces were clean and free of almost all attachments, which suggested cutting of links by free radicals degradation and dissolution of the end of polymer chains. Irradiation caused smoothness on the fiber surfaces, and fuzziness at the edges, which were signs of degradation by free radicals produced by electron beam in the sample. Scanning electron microscopy images showed that as irradiation doses (500 compared to 250) increased, the distance between the layers of the bagasse fibers increased and in some places, such as edges, scaling with high folds observed. These folds and fractures resulted in cavities in plant tissue. In 500 kGy irradiated sample, topography and folded were the regular track and waveform with high fractures near the edges.

There is limited information about the effects of electron beam irradiation on fiber morphology and structure of sugarcane bagasse, but there is some works on lignocellulosic decomposition. It has been clearly indicated that, when a sample exposed to irradiation, free radicals are formed within lignocellulose molecules (Von Sonntag, 1980). In this condition, cleavage of bonds between cellulose and lignin occur. These radicals could also cause degradation of lignocellulose via certain reactions. At high irradiation dosages, the cleavage reactions were duplicated and resulted in an increase of glycosidic bonds breakage (Von Sonntag, 1980).

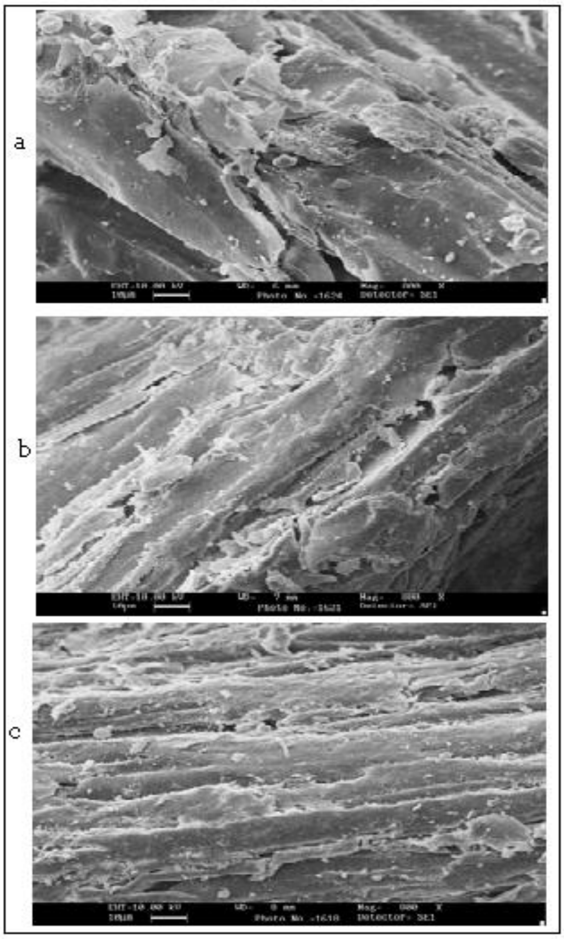

Fig. 1. The 800-times magnified scanning electron microscopy (SEM) images of untreated bagasse (a) and irradiated at doses of 250 (b) and 500 kGy (c).

Scanning electron microscopy images of untreated but incubated bagasse for 6 h (Fig 2 a) and also 250 kGy irradiated bagasse that incubated in the rumen for 6 h (Fig 2 b) and 12 h (Fig 2 c) are shown in Fig 2. The surface topography of untreated bagasse which incubated for 6 h in the rumen is highly rugged and elevated like a volcanic crater. This means that fibrolytic bacteria cannot colonize in the wide extent and degrade the fibers in the untreated bagasse. Irradiation resulted in the clean layers with smooth topography in the surface of particles. The higher cavity and pores in the fiber structure of irradiated samples resulted in the higher colonization and degradation. As seen in the Fig 2 b and c (irradiated samples), there were many cavities and multi-layer features compared with untreated sample (Fig 2 a). In addition to the increase in number of cavities by 250 kGy irradiation, the diameter and depth of the cavity was larger in 12 h incubated bagasse then 6 h as compared with untreated bagasse. This result showed that microorganisms had higher activity in longer period of incubation.

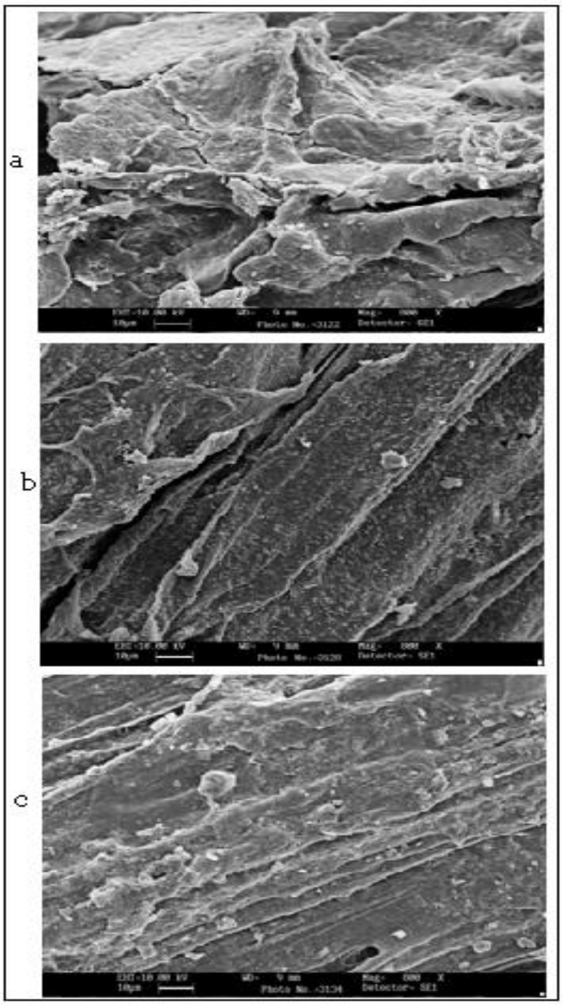

Fig. 2. Scanning electron microscopy images of untreated but incubated bagasse for 6 h (a) and 250 kGy irradiated bagasse that incubated in the rumen for 6 h (b) and 12 h (c).

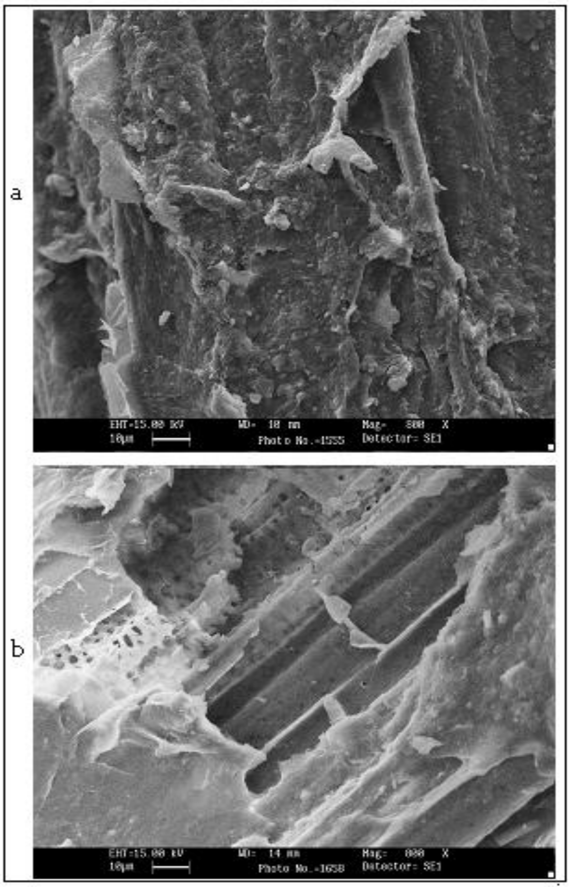

Fig. 3. Scanning electron microscopy images of 500 kGy irradiated bagasse that incubated in the rumen for 6 h (a) and 12 h (b).

Scanning electron microscopy images of 500 kGy irradiated bagasse that incubated in the rumen for 6 h (Fig 3 a) and 12 h (Fig 3 b) are shown in Figure 3. As seen in these images, there was a higher activity of fibrolytic bacteria on the surface of fibers at dose of 500 kGy. In this irradiation dose, the penetration of microorganisms and also layer separation was higher than dose of 250 kGy. In addition small particles were not observed on the surface, but irregular fractures and folds observed. Microorganisms were also likely to influence the surface into the cell walls of the bagasse fibers and micro-fibrils of bagasse increased so that the surface looks as soft and puffy structure.

Conclusion

The current study provides evidence that electron beam irradiation could improve the fiber degradation characteristics of sugarcane bagasse for ruminants. The SEM images showed that many changes occur in the structure and morphology of fibers in the bagasse by irradiation which finally increase the attachment of bacteria to the lignocellulosic materials and through these change resulted in higher degradability. The data suggest that higher doses of electron beam irradiation (500 compared to 250 kGy) had higher impacts on the fiber structure and degradability of sugarcane bagasse in the rumen.

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